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CIRCADIAN RHYTHMS, SLEEP AND AUTONOMIC FUNCTION IN PROGRESSIVE SUPRANUCLEAR PALSY: CHARACTERISTIC FEATURES AND RECIPROCAL INTERACTIONS

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TABLE OF CONTENTS

INTRODUCTION

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Progressive supranuclear palsy (PSP) is a sporadic tauopathy, characterized by accumulation of tau isoform with four repeat sequences (4R-tauopathy) in several areas of the central nervous system. Neuropathological features include neurofibrillary tangles, neuropil threads, tufted astrocytes, oligodendroglial coiled bodies, neuronal loss and gliosis particularly in subcortical structures (i.e. basal ganglia, subthalamic nucleus and brainstem), and later in cortical areas (mainly frontal and parietal) and cerebellar structures(Kovacs, 2017).

The main clinical phenotype, also called Richardson's syndrome as per initial description in 1964 and distinguished for the typical vertical supranuclear gaze palsy and postural instability with early falls, was subsequently revealed as being only one, although the most frequent, among several other possible clinical manifestations of PSP pathology(Williams *et al.*, 2005). The increasing number of descriptions of clinical heterogeneity of this condition led to the latest clinical diagnostic criteria(Höglinger *et al.*, 2017).

PSP falls under the broad category of the so called "atypical parkinsonisms", a group of neurodegenerative conditions characterized by parkinsonian signs in addition to other features, typically a poor response to medical treatment and poor prognosis. In certain cases, particularly at onset, atypical parkinsonisms may be misdiagnosed with Parkinson's disease (PD), which instead has a good response to medical therapy and a better prognosis. A detailed characterization of clinical features that mark one condition rather than another is fundamental to improve a precocious diagnosis especially in light of the upcoming trials with disease-modifying therapies.

Over the last few years, increasing attention has been focused on the "non motor symptoms" that are frequently associated, and sometimes represent characteristic features, of Parkinson's disease and atypical parkinsonisms. Some of these are important not only from a diagnostic point of view but also for treatment and prognosis.

For instance, a sleep disorder called REM sleep behaviour disorder is now considered a biomarker for a particular type of neurodegenerative process. Breathing disorders in sleep my represent poor prognostic factors. Circadian rhythms disruptions are more severe in patients affected with age-related neurodegenerative diseases and it has been suggested that they may not only be a symptom of neurodegeneration but possibly a potential risk factor(Leng *et al.*, 2019). A predominant, otherwise unexplained autonomic failure is listed among the mandatory exclusion criteria of PSP nevertheless several studies based on autopsy-proven PSP showed that autonomic features might be present also in PSP.

Therefore, there is a need for better characterization of non-motor aspects, particularly autonomic nervous system involvement, sleep and circadian rhythms in PSP. This may help to enhance our understanding of this highly disabling neurodegenerative disease including patients' needs and possibly be of value for clinicians in the differential diagnosis among parkinsonian syndromes.

Sleep and the autonomic nervous system are closely related from both biological and clinical perspectives. Central nuclei of the central autonomic network are closely connected to neural pathways located in the brainstem and basal forebrain involved in the regulation of the wake-sleep cycle. Several autonomic functions are physiologically modulated according to the wake and sleep stages and indeed sleep itself may be considered one of the most highly integrated autonomic functions. Hence, several neurological conditions that are associated with autonomic dysfunction as part of the same pathophysiological process, also present sleep disorders resulting from common mechanisms or as a direct consequence. On the other hand, sleep disorders may in turn alter the sympathetic/parasympathetic balance.
For instance, orthostatic hypotension may be associated with supine hypertension, which is prominent during night-time with prolonged recumbency. Similarly, nocturia resulting from urinary dysautonomia is one of the most common nocturnal complaints among affected people. Conversely, restless legs syndrome, periodic limb movements and obstructive sleep apnoea are known to increase sympathetic modulation in the general population and are risk factors for cardiovascular comorbidities such as hypertension. REM sleep behaviour disorder is associated with sympathetic failure. These alterations are important from the pathophysiological point of view and for the possible therapeutic implications. Moreover, they may have long-term consequences, especially in terms of cardiovascular and cerebrovascular comorbidity, and therefore need to be addressed.

The aims of this study were to describe and evaluate the association between sleep, the circadian system and autonomic function in a cohort of patients with PSP.
The study was approved by the Ethics Committee of Area Vasta Emilia Centro (reference number 18141) and performed in accordance with the Declaration of Helsinki.

Section I

SLEEP-WAKE CYCLE IN PROGRESSIVE SUPRANUCLEAR PALSY

BACKGROUND

Patients affected with movement disorders are known to frequently present various types of sleep abnormalities which have a significant impact on quality of life and disease progression. Moreover, specific patterns of sleep impairments or a particular sleep disorder may assist in the diagnostic process as long as it uniquely characterizes a disease over another. However, if not specifically addressed by direct questioning or testing, these aspects regarding sleep may often be neglected.

An increased awareness of sleep-wake cycle disorders and how they manifest in different movement disorders is essential to improve clinical practice.

Even though progressive supranuclear palsy (PSP) is actually one of the main conditions to be considered in the differential diagnosis of more common disorders like Parkinson's disease (PD) and other atypical parkinsonisms such as multiple system atrophy (MSA), studies evaluating sleep in PSP are scanty and heterogeneous, especially compared to the amount of literature available on sleep in PD and MSA.

Self-reported sleep problems

Sleep complaints were evaluated by means of different questionnaires or structured interview. Colosimo et al. found sleep disturbances (REM sleep behaviour disorder, insomnia, excessive daytime sleepiness and restless leg syndrome) investigated by structured interview) in 77% of PSP patients(Colosimo *et al.*, 2010). Similarly, Reimann and colleagues, also using a structured interview, reported a prevalence of 89% for sleep domain problems, with snoring as the most frequent symptom (52%)(Reimann *et al.*, 2010). Sleep/fatigue problems were reported by 83-100% of PSP patients on the Non-Motor Symptom Scale (NMSS)(Ou *et al.*, 2016; Radicati *et al.*, 2017; Chaithra *et al.*, 2020). Poor sleep quality defined as a Pittsburgh Sleep Quality Index (PSQI) > 6 was found in almost half of PSP patients(Gama *et al.*, 2010). Mean scores on PSQI and NMSS were higher in PSP than PD(Bhalsing *et al.*, 2013; Matsubara *et al.*, 2018) respectively. Frequent nocturnal awakenings were significantly more common in PSP than healthy controls, however only few patients were aware of such problem(De Bruin *et al.*, 1996). Interestingly, PSP patients tended to rate their sleep better than what PD patients did, even though objective sleep parameters on polysomnography (PSG) were significantly worse in PSP than PD, suggesting a lack of awareness(Sixel-Döring *et al.*, 2009).

In summary, sleep complaints seem considerable and reported in more than half of the patients in all studies. These studies are limited by the different methodologies used to investigate sleep disturbances and the relatively low sample. However, few studies that compared results of questionnaires and objective sleep measures suggested that PSP may lack of awareness of their sleep problems, therefore questionnaires and scales, as well as sleep history, may not be a reliable tool to properly assess sleep in this context.

Sleep structure

Sleep architecture in PSP was described in few studies that performed PSG.

Gross and colleagues in 1978 studied 4 patients with PSP by means of PSG and compared results with controls(Gross *et al.*, 1978). The Authors found a reduced total sleep time (TST) with an increased number and duration of awakenings. NREM sleep stage 1 was significantly more represented and stage 2 was characterized by poorly formed and infrequent spindles. REM sleep percentage was reduced.

Subsequently, Aldrich et al assessed 10 PSP patients and found that TST and sleep efficiency were significantly reduced compared to normative values and sleep fragmentation was due to a high number of awakenings and delayed sleep onset(Aldrich *et al.*, 1989). The same Authors reported a reduced representation of stage 2 and higher of stage 3 likely attributable to increased amounts of slow EEG activity and absent/poor representation of sleep spindles. REM stage percentage was significantly reduced compared to normative values but with normal latency and number of episodes. Sleep structure seemed to deteriorate with disease progression as marked by correlation of sleep parameters with motor scales.

Sleep efficiency and TST were reduced and wake after sleep onset (WASO) increased also in another small study (6 patients)(Montplaisir *et al.*, 1997). Other significant differences with controls were an increased stage 1 percentage, reduced REM percentage, and infrequent spindles.

Arnulf and coworkers studied 15 PSP patients finding a comparable TST between PSP and control group, although sleep efficiency was lower (without reaching statistical significance) and WASO was significantly increased. In this study, stage 1 representation was increased whereas REM sleep stage was reduced and presented with increased latency(Arnulf *et al.*, 2005). In the same study, a comparison between PSP and PD was also performed. PSP had reduced sleep efficiency compared to PD (but without reaching statistical significance) and similar REM sleep percentage(Arnulf *et al.*, 2005).

Sixel-Doring et al. and Nomura et al. compared the results of 20 PSG of PSP patients with those of 20 and 93 PSG of PD patients respectively (Sixel-Döring *et al.*, 2009; Nomura *et al.*, 2012). In both studies PSP showed lower sleep efficiency compared to PD. NREM stage 2 was less represented in PSP compared to PD in only one study(Sixel-Döring *et al.*, 2009). The percentages of REM stage were similar between PSP and PD in both studies.

Finally, Walsh and colleagues, in their study involving 19 PSP and 16 controls, showed that PSP had a reduced sleep efficiency, TST, NREM 2 %, NREM 3 % and REM %, as well as increased WASO and REM latency(Walsh *et al.*, 2017).

Two additional case reports described similar sleep findings(Di Trapani *et al.*, 1991; Lee, 1991).

In summary, the most consistent findings across these studies were a reduced sleep efficiency and TST with frequent awakenings and increased WASO in PSP. Regarding sleep stages, several studies reported poor representation of K-complexes and spindles and a significantly reduced percentage of REM sleep compared to normative/control values.

Compared to PD, PSP seems to present a reduced sleep efficiency and similar REM sleep percentage.

REM sleep without atonia and REM sleep behaviour disorder

REM stage has gained a lot of attention, particularly with regard to the occurrence of absence of the physiological atonia (REM without atonia, RWA) and REM sleep behaviour disorder (RBD).

RBD is a REM sleep parasomnia characterised by abnormal dream-enacting behaviours emerging during REM stages and associated with RWA. The patient acts the content of their dreams, which is usually violent and distressing (for example, the patient dreams a situation in which they are attacked or chased by animals). Clinically the patient manifests elementary motor activity such as twitching and jerking, followed by more structured, seemingly purposeful and usually violent movements such as punching, kicking, grabbing, etc., associated with vocalization. The patient is easily awaken by the dream and is able to recall its content. RBD may lead to injuries like falling out of bed whereas more complex behaviours such as walking outside the bedroom do not usually happen. Seldom, the content is pleasant and the patient may laugh, sing and gesture.

Confirmation of RBD diagnosis requires PSG that shows persistent muscle tone activation during REM stages (i.e. RWA) along with demonstration of episodes (on video, if available), or a characteristic clinical history of dream enactment(Berry *et al.*, 2017).

RBD may be idiopathic or associated with another neurological disorder, particularly neurodegenerative parkinsonisms of the alpha-synucleinopathy type (i.e. PD, MSA, dementia with Lewy bodies), for which it is considered a marker. In addition, RBD is known to predate the onset of motor symptoms in several cases, and therefore has been extensively investigated as a potential premotor symptom of neurodegenerative diseases.

The occurrence and the diagnostic/prognostic role of RBD in PSP still need to be clarified. As a matter of fact, studies have reported somewhat conflicting results. As mentioned earlier, RBD has long been considered a marker of alpha-synucleinopathies.

Olson et al. reviewed the medical records of 93 patients with a diagnosis of RBD confirmed with PSG(Olson *et al.*, 2000). Of these, only 1 (2%) had a diagnosis of PSP while more common diagnoses were PD (47%), MSA (26%) or dementia without parkinsonism (13%).

Boeve et al. systematically interviewed about possible RBD all patients referred for parkinsonism or cognitive impairment, and those patients with suspected RBD subsequently underwent PSG(Boeve *et al.*, 2001). Among 398 patients included in this study, 5 had PSP. Of these, only 1 reported a history of RBD which was not however confirmed on PSG. On the contrary, a history of RBD was reported in 77/120 patients with MSA/PD/DLB, and confirmed in 45 on PSG. The study concluded that if RBD was present in a patient with a dementing or parkinsonian disorder it was highly suggestive of a synucleinopathy. However it was not a sensitive feature to identify a synucleinopathy.

Both these studies, based on large cohorts of patients with RBD, supported the hypothesis that RBD is associated far more frequently with synucleinopathies than tauopathies. However it should be noted that they included a small sample of PSP cases which may represent a limitation.
Other studies focusing on PSP cohorts confirmed the absence/rarity of RBD in PSP. RBD was never reported in the study by Cooper et al. who interviewed 10 PSP patients (2 with pathological confirmation) and bed partners(Cooper and Josephs, 2009). Similarly, RBD was never found in the PSG studies on 6(Montplaisir *et al.*, 1997) and 20(Nomura *et al.*, 2012) PSP patients.
Conversely, other researchers investigating PSP by means of PSG detected RBD in a higher percentage of cases (2/15, 13%(Arnulf *et al.*, 2005); 7/20, 35%(Sixel-Döring *et al.*, 2009)). Likewise, studies using clinical interviews found similar prevalence (20%(Diederich *et al.*, 2008), 37%(Munhoz and Teive, 2014)). Therefore, these studies suggest that RBD may actually be more frequent in PSP than previously thought and should not preclude the diagnosis of a tauopathy.

RBD aside, RWA itself seems to be present rather frequently. Arnulf et al found 33% of RWA (% of total REM sleep) in PSP which was similar to that of PD(Arnulf *et al.*, 2005). In the study by Sixel-Doring et al., RWA percentage (determined using the same method by Arnulf et al.) was found in 85% of PSP patients (vs 95% PD), however the total amount of RWA was significantly lower in PSP (14.5%) compared to PD (44.6%)(Sixel-Döring *et al.*, 2009). Nomura et al found RWA in 20% of PSP patients and a mean RWA % of 10.5, both values were significantly smaller compared to those of the PD group(Nomura *et al.*, 2012). On the contrary, the physiological atonia was preserved in former studies performed on smaller samples(Gross *et al.*, 1978; Montplaisir *et al.*, 1997).
In conclusion, most data seem to indicate that RWA might be present in PSP although in less quantity than PD. Indeed a recent study quantitatively analysed RWA in 46 adults with cognitive impairment (20 probable synucleinopathy and 26 probable non-synucleinopathy – i.e. Alzheimer disease, frontotemporal dementia) and the results showed that elevated submentalis (but not anterior tibialis) RWA distinguished the two groups (being higher in the synucleinopathy one) even in the absence of clinical dream enactment(McCarter *et al.*, 2020). Therefore, the quantity rather than the presence/absence of RWA might indeed being a more characteristic feature of neurodegenerative disorders with different aetiologies.

Restless legs syndrome and periodic leg movement of sleep

Restless legs syndrome (RLS) is a sensorimotor disorder characterized by an urge to move the legs usually accompanied by uncomfortable and unpleasant sensations(Allen *et al.*, 2014). This urge and the associated symptoms begin during periods of rest and ameliorate with movement (walking, stretching) as long as the activity continues. Importantly, a strong circadian variation in symptoms intensity is a unique diagnostic feature of RLS, whose manifestations are most pronounced in the evening and night, with gradual

improvements toward early morning and during the day. This last feature might be lost eventually in cases of very severe, usually long standing RLS. The diagnosis of RLS is clinical, and diagnostic work-up is limited to blood tests to exclude secondary causes such as iron deficiency anaemia and uraemia(Trenkwalder *et al.*, 2018).

Approximately 80-90% of patients with RLS also have periodic limb movements of sleep (PLMS)(Allen *et al.*, 2014), that are repetitive, stereotyped, involuntary movements of the legs mainly manifesting as flexion of the ankle and toe (sometimes also flexion of the hip and knee). PLMS are quantified with EMG recording of the anterior tibialis muscle during polysomnography(Berry *et al.*, 2017). A leg movement (LM) is scored when an activity of at least 8 microV amplitude and duration between 0.5 and 10 seconds is recorded. A series is constituted by at least 4 LMs occurring consecutively within 5 to 90 seconds. This periodicity is typical of PLMS and the number of LMs in series per hour, expressed as the PLMS index gives a measure of the intensity of this phenomenon. Usually, a PLMS index > 10 is considered clinically significant.

RLS is another condition frequently encountered in PD. Few studies explored this feature in PSP.

Gama et al. found an unexpectedly high prevalence of RLS (diagnosed according to IRLSSG criteria) in PSP (57%), even higher than PD and MSA(Gama *et al.*, 2010). In the PSP group, RLS was associated with reduced sleep duration and sleep efficiency. The Authors suggest that RLS might be an under-recognized condition affecting PSP patients' sleep.

Conversely, in the study by Bhalsing et al., only one out of 27 PSP patients had RLS (which was instead more common in the PD group)(Bhalsing *et al.*, 2013). Possible reasons for this discrepancy could be related to younger age and shorter disease duration in this work compared to the previous one by Gama et al.

Similarly, Matsubara et al did not find RLS in PSP, even though 9% fulfilled the criteria for leg motor restlessness (an urge to move the legs that did not fulfil the four essential features of RLS)(Matsubara *et al.*, 2018). Instead, RLS was found in 13% of PD patients and 6% of MSA patients. The Authors hypothesize that frontal lobe dysfunction typical of PSP may contribute to a lack of awareness of restlessness in this neurodegenerative disorder.

A single case report illustrated a case of PSP with severe RLS that responded to dopaminergic medication (rotigotine patch)(Moccia *et al.*, 2015).

In the study by Chaithra and coworkers, 14.5% of PSP patients experienced an urge to move the legs or restlessness in legs that improved with movement when they were sitting or lying down inactive (as per the NMSS)(Chaithra *et al.*, 2020).

In summary, most studies demonstrated that RLS is seen in a minority of PSP patients.

PLMS were scored in few PSG studies. In the case series by Aldrich et al 2 out of 10 patients presented PLMS(Aldrich *et al.*, 1989). Arnulf et al. found a PLM index in PSP similar to that of PD and healthy controls,

although more subjects with PSP (7) had a PLMI > 25 (compared to 5 subjects with PD and 2 healthy controls)(Arnulf *et al.*, 2005). Conversely Sixel-Doring et al. reported that PSP had a significantly higher PLMS index compared to PD and 19 (out of 20) PSP had a PLMI > 25 (compared to only 15 PD patients) (Sixel-Döring *et al.*, 2009). Finally, in the study by Walsh et al, 52% of PSP had a PLMI > 25(Walsh *et al.*, 2017).

In summary, PLMS seem to occur frequently and more severely in PSP than PD. Figures obtained from these few and heterogeneous studies do not seem to show a correlation between PLMS and RLS in PSP.

Sleep-related breathing disorders

As mentioned above, snoring is a frequent complaint in PSP(Reimann *et al.*, 2010). The relevance of snoring is related to the possible breathing disorder of which it may be a harbinger, that is obstructive sleep apnea (OSA).

One study that evaluated the risk of OSA by means of the Berlin Questionnaire found that only 2 out of 14 (14%) PSP patients were considered at risk(Gama *et al.*, 2010). The risk of OSA was associated with sleep disturbance and diurnal dysfunction as detected by the Pittsburgh Sleep Quality Index. In addition, risk of OSA correlated with age. Compared to other parkinsonisms (PD and MSA), OSA risk was lower in PSP.

Regarding studies that objectively assessed sleep in PSP, Aldrich et al. firstly reported that 2 out 10 patients presented sleep apnea (1 central and 1 obstructive)(Aldrich *et al.*, 1989).

De Bruin and colleagues performed overnight oxygen saturation monitoring and spirometry in 11 PSP patients(De Bruin *et al.*, 1996). They found that nocturnal oxymetry was within normal limits in all cases whereas spirometry could not be performed properly by the patients. These results suggested that PSP may have a supranuclear impairment of (voluntary) respiratory control whilst maintaining automatic and limbic emotional control of breathing.

Arnulf et al. found a mean AHI of 25 in PSP, with 9 patients (60%) having a AHI > 15(Arnulf *et al.*, 2005). Nor the mean value nor the frequencies differed significantly between PSP, PD and control groups.

In the study by Sixel-Doring et al., 11 PSP patients (55%) presented an AHI > 5(Sixel-Döring *et al.*, 2009). Similarly to the previous study, there was no significant difference with PD values. Moreover, no significant correlation could be detected between AHI>5 and the PDSS item 15 investigating excessive daytime sleepiness.

Finally, Walsh and coworkers found that 32% PSP patients had an AHI > 10, which was more common than in the control group (12.5%)(Walsh *et al.*, 2017). However the controls had been previously screened for OSA as an exclusion criteria. Additionally, 2 PSP already had a diagnosis of OSA and wore their CPAP during the test which resulted in a AHI < 10.

In summary, OSA is not uncommon in PSP and may affect approximately 50% of patients. It is not clear however whether the prevalence and severity is increased with respect to the general population or other

neurodegenerative diseases like PD, because very few studies addressed this topic and provided conflicting evidence.

Sleep-wake cycle in PSP

Sleep is tightly linked to wake in a pattern that possibly represents the strongest circadian cycle of the human body. As a consequence, poor night-time sleep invariably leads to repercussions on daytime wakefulness. Nevertheless, neurodegenerative pathology affecting specific areas of thalamus and brainstem as it happens in PSP may also have an influence on the daytime part of the cycle. In other words, the consequences on daytime vigilance after a poor night-time sleep may not be the same as in a healthy human subject.

Excessive daytime sleepiness frequently results from poor sleep overnight. The Epworth Sleepiness Scale (ESS) is a validated questionnaire to measure the subject's dozing probability during hypothetical daytime situations. In the study by Gama et al., 43% of PSP patients had excessive daytime sleepiness (defined as an ESS score > 10)(Gama *et al.*, 2010). On the contrary, excessive daytime sleepiness did not seem a prominent feature of PSP in the work by Bhalsing et al., where mean ESS score for PSP was 7.3 ± 3.6 (Bhalsing *et al.*, 2013). Similar results were obtained by Matsubara et al (mean ESS score 6.4 ± 10)(Matsubara *et al.*, 2018) and Arnulf et a. (6.7 ± 5.0, 2 out of 15 patients had a ESS > 10)(Arnulf *et al.*, 2005). Chaithra et al., using the NMSS, found that approximately 37% of PSP patients reported dozing off or falling asleep unintentionally during daytime activities and 66% had fatigue or lack of energy limiting daytime activities(Chaithra *et al.*, 2020).

Further insights regarding wakefulness in PSP come from studies employing instrumental tests, particularly the Multiple Sleep Latency Test (MSLT) which assesses the propensity to fall asleep across the day. Montplasir et al. were able to assess by means of MSLT 3 out of 6 PSP patients included in their study(Montplaisir *et al.*, 1997). The results showed high intersubject variability and the Authors could not draw any conclusion.

Arnulf et al. performed PSG and MSLT in 15 PSP patients(Arnulf *et al.*, 2005). Four patients had abnormal (< 8 min) mean sleep latencies. Such patients had an arousal index similar to those with normal daytime mean sleep latencies, however slept longer during the night (longer TST). Therefore, since patients with excessive daytime sleepiness had similar sleep fragmentation but longer nocturnal sleep than those with normal alertness, the Authors suggested that sleepiness in PSP may be caused by a primary central hypersomnia. Subsequently, Walsh and colleagues performed a similar study involving 19 PSP(Walsh *et al.*, 2017). Compared to sex- and age-matched controls, PSP patients took longer to fall asleep and this was confirmed even after excluding the patients that remained awake throughout the MSLT assessment. In contrast to such MSLT results, PSP reported higher levels of sleepiness compared to controls based on the Stanford Sleepiness Scale. In conclusion, this study provided evidence that PSP had great overnight sleep disruption

(reduced TST, frequent nocturnal awakenings) but did not sleep during the day and did not show shorter sleep latencies to sleep on MSLT compared with controls. Moreover, the objective evidence of low propensity to sleep during daytime was incongruent with the subjective sleepiness reported by the patients. The Authors hypothesize that subjects with PSP present a disruption of sleep/wake regulation mechanisms, particularly they do not respond appropriately to the overnight sleep debt (by napping during daytime) suggesting an impaired sensitivity to the homeostatic sleep drive with an increased arousal across the 24-h.

Aim of this study was to describe the characteristics of the sleep-wake cycle recorded by 24-h PSG under controlled environmental conditions in a sample of patients with PSP.

MATERIALS AND METHODS

Participants

Fifteen patients with PSP diagnosed according to international criteria(Höglinger *et al.*, 2017) were included in this study. Two patients were recruited retrospectively and 13 prospectively. None of the patients had concomitant severe medical conditions that could have affected study results. All patients were admitted to the hospital to perform the study protocol. All patients gave written informed consent to participate in the study.

Sleep questionnaires and scales

Sleep quality was assessed by the PSQI (Buysse *et al.*, 1989; Curcio *et al.*, 2013). This questionnaire consists of seven components investigating major aspects of sleep over the previous 4 weeks: (1) subjective quality of sleep; (2) sleep onset latency; (3) sleep duration; (4) habitual sleep efficiency; (5) the presence of sleep disturbances; (6) the use of hypnotic or sedative medications; and (7) the presence of daytime dysfunction as a consequence of poor over night sleep. An individual with a PSQI global score of 5 ore more is considered a poor sleeper.

Daytime sleepiness was evaluated by the ESS (Vignatelli *et al.*, 2003). This questionnaire comprises enquiries about the subject's propensity of dozing off in eight hypothetical real-life situations over the preceding months. The ratings range from zero (no probability) to three (high probability). An ESS score of more than 10 indicates excessive daytime sleepiness.

Restless legs syndrome (RLS) was established according to the criteria defined by the International RLS Study Group (Walters, 1995): (1) desire to move the limbs usually associated with some definable discomfort; (2) motor restlessness; (3) worsening of at rest with at least partial and temporary relief by activity; (4) worsening of symptoms in the evening or at night. In case the RLS criteria were satisfied, the interview continued with questions regarding additional features such as positive family history. The

severity of RLS was further assessed by the RLS rating scale (Walters *et al.*, 2003) which consists of 10 multiple choice questions. The higher the score, the more severe is RLS (mild: scores from 0 to 10; moderate: 11 to 20; severe: 21 to 30; very severe: 31 to 40).

Patients and caregivers were interviewed using a semi-structured questionnaire to check the occurrence of RBD according to diagnostic criteria (American Academy of Sleep Medicine, 2014). When criteria were confirmed, a structured and validated questionnaire (Scaglione *et al.*, 2005) focusing on RBD features (clinical characteristics, duration and time of onset during the night, frequency, occurrence of sleep related injuries) over the last 12 months was administered.

48-h polysomnography

Sleep-wake cycle was monitored by an ambulatory polygraphic recorder (Albert Grass Heritage®, Colleague TM PSG Model PSG16P-1, Astro-Med, Inc, West Warwick, RI, USA or Neurofax Electroencephalograph EEG-1200, Nihon-Kohden Corp., Tokyo, Japan). Polygraphic recording included electroencephalogram (EEG: Fp_1-F_3, F_3-C_3, C_3-P_3, P_3-O_1, Fp_2-F_4, F_4-C_4, C_4-P_4, P_4-O_2, C_z-P_z), right and left electrooculogram (EOG), electrocardiogram (EKG), electromyogram (EMG) of the mylohyoideus, intercostalis, extensor carpi (left and right) and anterior tibialis (left and right) muscles, thoraco-abdominal breathing (strain gauge) and video-synchronized recording. Oronasal airflow sensor and pulse oximetry were applied after the subject finished dinner and maintained for the night-time.

During the study, subjects were allowed to sleep ad libitum, living in a temperature (24 ± 1 °C) and humidity (40-50%) controlled room, lying in bed except when eating, in a light-dark schedule (dark period: 23:00 - 7:00). The subjects were placed on a 1.800 kcal/day diet, divided into three meals (8:00, 12:00, 18:00) and three snacks (10:00, 16:00, 23:00). From midnight preceding the monitoring, subjects were instructed to avoid alcohol and caffeinated beverages and to abstain from smoking

The 24-h sleep-wake cycle was visually scored in 30-second epochs according to the American Academy of Sleep Medicine criteria (Berry *et al.*, 2017) as stages 1, 2 and 3 NREM sleep and REM sleep. The light-off period was considered from 23:00 to 7:00 (scheduled dark period); however, if the patient fell asleep before 23:00 or woke up after 7:00 the reference time was changed accordingly to include the actual sleep time of the patient. Time in bed (TIB), total sleep time (TST), sleep efficiency (SE: TST/TIB * 100), duration and percentage referred to TST of each NREM and REM sleep stage were calculated for each subject over the 24 hours (dark period of the two consecutive nights and light period of the second day of recording). Latency to sleep onset was estimated only for patients that fell asleep after 23:00. We further computed latency to REM sleep, number of REM sleep episodes, percentage of tonic and phasic muscle activity in REM sleep, duration and percentage of wake after sleep onset (WASO), number of awakenings, arousal index (AI: number of arousals/hour of sleep), PLMS index (PLMSI: number of periodic limb movements/hour of sleep), number of obstructive, central and mixed apnoea and hypopneas, apnoea-

hypopnea index (AHI: number of obstructive and mixed apnoea and hypopnea/hour of sleep) and oxygen desaturation index (ODI: number of oxygen desaturations ≥ 3%/hour of sleep) according to AASM criteria over the two consecutive nights.

Statistical analysis

Statistical analyses were performed with SPSS Statistics (version 25). Normality was checked for each variable with the Shapiro-Wilk test. Comparisons were performed with Student *t*-test for normally distributed data and Mann-Whitney test for not normally distributed data. Statistical significance was set at $p \leq 0.05$.

RESULTS

Two male patients were not able to perform the study protocol because during the first night removed most of the recording set and were therefore excluded. The remaining 13 patients completed at least 24 hour of recording and their demographics and clinical characteristics are summarized in **Table 1**. Mean age of the sample was 75 ± 3 years, and more than half of the subjects were females (62%). Disease duration was 5.3 ± 1.7 years. The severity of disease was estimated by the PSPRS (50.5 ± 10.8), H&Y stage (3.8 ± 0.7) and MMSE (25.0 ± 2.7). Eight patients were treated with dopaminergic medications (6 with levodopa, 2 with levodopa + dopamine agonist). Two patients were treated with sedatives and six with antidepressants that for ethical reasons could not be discontinued.

Mean PSQI was 10.4 ± 6.3. Nine patients (70%) had an overall score > 5 (poor sleepers). Mean ESS score was 5.9 ± 3.6. Only 2 patients reported pathological excessive daytime sleepiness (overall score > 10). Six patients (46%) satisfied the criteria for RLS with a mean gravity score of 12 ± 2. At RBD screening no patient was suspected of having RBD but two were reported of having sleep talking.

Sleep parameters of the PSP patients over the dark and light periods of the last 24 hours of recording are reported in **Table 2**. Overall PSP patients slept less than 5 hours/night (mean TST 286.8 ± 118.3 min). Mean sleep efficiency was 53%. Night-time sleep was interrupted by frequent awakenings (approximately 40) with prolonged wake periods (mean WASO 231.9 ± 137.2 min). Eight patients fell asleep before the light-off stimulus at 23:00. For the remaining, sleep latency (expressed as median (interquartile range)) was 39 (49.5) min. All sleep stages were represented in all patients but 1 that did not reach REM stage. The mean percentage of NREM sleep stage 1 was 24.2 ± 13.7 %, NREM sleep stage 2 was 45.1 ± 9.1 %. Slow wave sleep (NREM stage 3) was 24.2 ± 14.7 % and REM sleep was 9.9 ± 8.7 %. REM onset latency was prolonged (> 90 min) in 9 out of 12 patients. Only one patient presented tonic activity during REM sleep that was

associated with motor activity and vocalizations compatible with RBD. It should be noted that the patient that did not reach REM stage during night sleep had a prolonged nap (184 min) during the first day of recording where REM sleep was reached after 158.5 min and was characterized by tonic activity and motor/vocal activity (i.e. RBD). After this nap, this patient never reached REM sleep stage again for the rest of the recording (nor in the first nor second night and neither during the second day of recording). Since data from the last 24 hours of recording are reported for all patients, this particular nap was not showed in the tables but it was considered for the diagnosis of RBD for this particular patient. In summary, 2 out of 13 patients had PSG confirmed RBD. Ten patients presented increased phasic activity during REM stage (mean % of phasic RWA 27.9 ± 35.7, median 16.2 (31.9)). There were no significant differences in REM sleep %, duration and % of RWA between patients with and without antidepressant treatment at the time of the recording ($p > 0.05$ in all comparisons).

Ten patients presented PLMS, of these 8 had a pathological PLMSI (>10) and 7 had PLMSI > 25. There were no significant differences in PLMSI, TST, SE, WASO, duration of each sleep stage, arousal index and number of awakenings between patients with and without RLS ($p > 0.05$ in all comparisons). Similarly, there were no significant differences in TST, SE, WASO, duration of each sleep stage, arousal index and number of awakenings between patients with PLMS > and < 25 ($p > 0.05$ in all comparisons).

The evaluation of the AHI was limited by the fact that oronasal and oxygen saturation were not recorded in the two retrospective patients and further 3 patients removed the oronasal sensor during the night. Therefore, AHI was available for 8 patients. AHI was normal for 4 patients, and within 5-15 for the remaining 4 patients. The 3 patients that removed oronasal sensor overnight (and for whom it was not possible to calculate AHI) had an ODI of 85, 25 and 3.

During the light period, all patients had one or more naps with a sleep duration of 42 (57) min (median (interquartile range)). Only 3 patients slept more than 60 min. There were no significant differences in nighttime parameters of TST, duration of each sleep stage, arousal index and number of awakenings between patients that slept more than 60 min and those that slept less than 60 min during daytime ($p > 0.05$ in all comparisons). During daytime, light sleep (NREM stage 1 and 2) represented the majority of sleep.

Table 1_Demographics and clinical characteristics of PSP patients

N	13
Age (years)	75 ± 3
Females (n, %)	8 (62%)
Disease duration (years)	5.3 ± 1.7
Hohen &Yahr stage	3.8 ± 0.7
PSPRS	50.5 ± 10.8
MMSE	25.0 ± 2.7
Dopaminergic therapy	
Levodopa	6 (46%)
Levodopa + dopamine agonist	2 (15%)
LEDD (mg)[a]	250 (100-680)
Other therapy	
Sedatives	2 (15%)
Antidepressants	6 (46%)

Legend: *N* = number of subjects; PSPRS = progressive supranuclear palsy rating scale; MMSE = mini mental state examination; LEDD = levodopa equivalent daily dose.
Data are expressed as mean ± standard deviation or number of subjects (%).

Table 2_Sleep parameters of PSP patients

Dark period		
TST (min)	286.8 ± 118.3	*261 (138.5)*
Sleep efficiency (%)	52.9 ± 20.1	*54.1 (23.3)*
Sleep latency (min)	66.3 ± 76.9	*39 (49.5)*
WASO (min)	231.9 ± 137.2	*204 (105.5)*
NREM 1 (%)	24.2 ± 13.7	*26.6 (19.9)*
NREM 2 (%)	45.1 ± 9.1	*43.2 (16.2)*
NREM 3 (%)	24.2 ± 14.7	*19.7 (21.7)*
REM (%)	9.9 ± 8.7	*10.8 (11.2)*
REM latency	151.3 ± 121.7	*118.0 (90.8)*
RWA tonic (%)	7.6 ± 26.3	*0 (0)*
RWA phasic (%)	27.9 ± 35.7	*16.2 (31.9)*
RWA tot (%)	27.9 ± 35.7	*16.2 (51.6)*
PLMSI	43.1 ± 45.5	*33.9 (67.9)*
AHI	8.1 ± 5.3	*5.2 (10.1)*
Awakenings (n)	37.7 ± 15.5	*37 (23)*
Arousal index	21.4 ± 7.8	*19.6 (9.3)*
Light period		
TST (min)	51 ± 46.9	*42 (57)*
NREM 1 (%)	54.2 ± 25.3	*50 (33.4)*
NREM 2 (%)	33.6 ± 20.9	*34.8 (30.5)*
NREM 3 (%)	11.9 ± 16.2	*4.7 (15.42)*
REM (%)	0.3 ± 1.1	*0 (0)*
Dark + Light (24 h)		
TST (min)	337.8 ± 131.9	*317 (172.5)*
NREM 1 (%)	26.3 ± 12.0	*28.0 (21.6)*
NREM 2 (%)	41.6 ± 14.9	*42.1 (17.6)*
NREM 3 (%)	23.6 ± 13.6	*21.5 (16.6)*
REM (%)	8.5 ± 7.2	*7.4 (9.3)*

Legend: TST = total sleep time; WASO = wake after sleep onset; NREM and REM stages are expressed as % of TST; RWA = REM sleep without atonia; PLMSI = periodic limb movements of sleep index; AHI = apnoea-hypopnea index (obstructive and mixed).
Data are expressed as mean ± standard deviation and *median (interquartile range)*.

DISCUSSION

This study evaluated the 24-h sleep-wake cycle in a cohort of PSP patients under controlled environmental conditions. Patients were studied in a room with fixed light/dark stimuli and meal schedule and were allowed to sleep ad libitum. This study design allowed, for the first time to our knowledge, the assessment of PSP patients' propensity to sleep as well as sleep characteristics over a complete sleep-wake cycle. There are several main findings from this study.

About 70% of PSP patients complained of poor sleep. This was mainly related to inability to fall asleep or maintain sleep. Excessive daytime sleepiness was not a major complain and only 2 patients (15%) had a pathological ESS score. At PSG, SE was markedly lower than normative values expected for this age range from our sleep laboratory (77% for males, 82% for females). PSP had reduced TST and increased WASO with frequent awakenings, which reflected the main patients' complaints. During daytime, median total duration of naps was 42 minutes with only 3 out of 13 patients (23%) sleeping more than 1 hour. Daytime naps were short and dispersed throughout the day and no patient completed a full sleep cycle. As a matter of fact, sleep during daytime was mainly represented by light sleep. Even when considering the total amount of sleep/24 h (summing the dark + light periods), median TST was 317 min (5.3 h) which is still lower than the recommended amount of sleep for the elderly(Watson *et al.*, 2015). Further insights could be derived by comparing TST of our PSP with those of 10 healthy and younger controls that performed the same protocol at out Institution and were previously published(Grimaldi *et al.*, 2010). Healthy controls slept approximately 334.8 min/night (dark period) and during the light period 56.95 min. Therefore, PSP slept less than healthy and younger controls during both night and day. While a reduction in sleep duration could be expected by aging, the same is not true for daytime naps that with aging tend to become longer as a compensating mechanism for the sleep loss overnight to maintain the total sleep requirement stable across the 24 hours(Chokroverty, 2017). Instead, these data suggest that PSP have a sleep loss during nighttime and do not compensate with sleeping during daytime, yielding to a condition of profound sleep deprivation across the 24 hours.

Compared to normative values for this age range of our sleep laboratory, PSP presented higher proportion of light sleep NREM stage 1 (n.v. 9.5% for males and 6.6% for females) and slightly lower NREM stage 2 (n.v. 55.5% for males and 52.2% for females), possibly reflecting a poor representation of sleep spindles and K-complexes. NREM stage 3 was increased in PSP (n.v. 1.4% for males and 10% for females), which is likely to be related to increased EEG slowing, while REM sleep duration was lower than expected (n.v. 17.70% for males, 19.50% for females).

Previous studies addressing sleep disturbances by means of questionnaires/interview found that approximately 77 – 100% of patients reported poor sleep or significant sleep problems(Colosimo *et al.*, 2010; Reimann *et al.*, 2010; Ou *et al.*, 2016; Radicati *et al.*, 2017; Chaithra *et al.*, 2020). Compared to previous studies using the PSQI, we found a higher mean score than Bhalsing et al. (7.2 ± 3.1)(Bhalsing *et al.*, 2013) and the percentage of poor sleepers was higher compared to the study by Gama et al (43%)(Gama *et al.*, 2010). This difference could be attributed to the younger population of patients in these two works compared to ours. In addition, Gama et al. used as a cut off for poor sleep a score higher than 6. The alterations of sleep structure observed in this study are in line with the few previous PSG studies(Gross *et al.*, 1978; Aldrich *et al.*, 1989; Montplaisir *et al.*, 1997; Arnulf *et al.*, 2005; Walsh *et al.*, 2017).
Regarding excessive daytime sleepiness, only 13 % of our patients had a pathological ESS, similar to the study by Arnulf et al.(Arnulf *et al.*, 2005). The mean ESS score was below 10 as in previous studies(Arnulf *et al.*, 2005; Bhalsing *et al.*, 2013; Matsubara *et al.*, 2018). Only Gama et al. reported excessive daytime sleepiness in a higher percentage of cases (43%)(Gama *et al.*, 2010).
In the present study, the lack of subjective complaint of daytime sleepiness is confirmed by the PSG recording. The patients, even though resting all day in a room without being engaged in any activity had only short naps of approximately 42 minutes in total. This is the first study addressing the whole 24-h sleep wake cycle in PSP by means of PSG. However two previous studies addressed the propensity to sleep during day by means of MSLT. Our results seem to confirm the findings by Walsh and colleagues, who found longer latencies to fall asleep during daytime despite poor nighttime sleep and hypothesized that PSP patients may present impaired sensitivity to the homeostatic sleep drive with an increased arousal across the 24-h(Walsh *et al.*, 2017). On the contrary, 4 out of 15 PSP patients in the study by Arnulf et al. had shorter sleep latencies and the same tended to sleep longer during night-time which suggested to the Authors that sleepiness in PSP was caused by primary central hypersomnia(Arnulf *et al.*, 2005). The reasons for the discrepancies among these studies are not entirely clear. The relatively small number of patients included in each study may represent a factor. The rarity of this condition and the complexity of these instrumental tests that are available only in tertiary referral centres limit the enrolment of patients and their compliance. Nevertheless further studies are encouraged in this setting to better elucidate this interesting topic.

Two patients (15%) had PSG proven RBD and 10 (77%) had increased phasic activity during REM sleep (median 16% of total REM). Therefore, even though RBD is considered a typical marker of synucleinopathies, this study shows that it can be found even in tauopathies. We did not have neuropathological confirmation for the diagnosis however these patients had Richardson's syndrome phenotype which is the one associated with the highest specificity for PSP pathology. In this cohort, the prevalence of RBD was 15%, which was similar to that found by Arnulf et al. (13%)(Arnulf *et al.*, 2005) and

slightly lower than the prevalence observed by Nomura et al. (20%) and Sixel-Doring et al. (35%)(Sixel-Döring *et al.*, 2009; Nomura *et al.*, 2012). Considering the results of all these PSG studies, RBD seems to occur in a minority (less than one third) of PSP patients. The frequency rather than the mere presence/absence may be the real discriminant factor between tauopathies and synucleinopathies. As a matter of fact, it is estimated that 30 to 50% of patients with PD have RBD and this figure is even higher (> 70%) for patients with DLB or MSA(Dauvilliers *et al.*, 2018; Giannini *et al.*, 2021). In addition, while isolated RBD is a known prodromal marker of synucleinopathies with an estimated conversion rate of 6.3 % per year and with 73.5 % converting after 12-year follow-up(Postuma *et al.*, 2019), there are no reports of prodromal RBD for PSP. Only one study recently addressed prodromal symptoms in a cohort of 50 PSP patients, finding that 2 patients reported dream enacting behaviour predating motor onset. However this study did not have PSG confirmation and therefore the value is limited(Painous *et al.*, 2020). This study also confirms previous findings regarding RWA in PSP(Arnulf *et al.*, 2005; Sixel-Döring *et al.*, 2009; Nomura *et al.*, 2012). We found similar small percentages of RWA in the majority of PSP patients. Therefore, in this case the quantity of RWA may characterize this feature in PSP. There were no differences in REM stage characteristics (including duration) between patients using antidepressants and those not treated, suggesting that disrupted REM sleep in PSP was independent of the potential effect of such medications.

About 6 PSP patients (46%) had RLS of moderate intensity and 8 (62%) had pathological PLMS. This study confirms the RLS prevalence observed by Gama et al.(Gama *et al.*, 2010) even though we did not observe a significant impact of RLS on sleep parameters. PLMS were present in more than half of PSP patients with a PLMSI > 25. The occurrence of severe PLMS in PSP confirms previous findings(Arnulf *et al.*, 2005; Sixel-Döring *et al.*, 2009; Walsh *et al.*, 2017). As for RLS, we did not observe a significant impact of PLMSI on sleep structure in PSP. Therefore, RLS and severe PLMS seem characteristic features of PSP sleep although they occur irrespectively of poor sleep structure. Nevertheless, further studies on this topic should be encouraged as PLMS may be treated and it would be interesting to evaluate the effect of dopaminergic treatment on PLMS in PSP and whether it provides an improvement on sleep.

In this study, 6 out of 11 patients (54%) had OSA of mild (4 patients), moderate (1 patient) and severe (1 patient) grade. This is in line with previous studies finding approximately similar percentages(Arnulf *et al.*, 2005; Sixel-Döring *et al.*, 2009; Walsh *et al.*, 2017). Instead, central apnoea were infrequent and observed only rarely in a minority of patients.

Pathophysiological mechanisms underlying the sleep disturbances observed in PSP still need to be clarified. Walsh and colleagues(Walsh *et al.*, 2017) proposed an interpretation involving an alteration of the homeostatic sleep drive by decreased slow wave activity induced by monoaminergic loss in the locus

coeruleus, dorsal raphe, tuberomammillary nucleus and cholinergic loss in the magnocellular nucleus and by basal forebrain cholinergic loss; in addition, galaminergic neuronal loss within the intermediate nucleus and GABAergic/glycinergic loss in the parafacial zone to induce increased wake after sleep onset. Another area that may be potentially involved in the sleep/wake disturbances in PSP is the pedunculopontine nucleus (PPN). The PPN contains cholinergic, GABAergic and glutamatergic neurons that are involved in control of locomotion and muscle tone but also in regulation of sleep-wake cycle and behavioural states by multiple connections with orexin neurons of the hypothalamus, tuberomammillary nucleus, dorsal raphe, locus coeruleus, laterodorsal tegmental nucleus and contralateral PPN(Benarroch, 2013). In particular, the PPN is critical for cortical arousal and transition between wakefulness and sleep. The PPN is known to be affected in PSP as in other parkinsonian disorders such as PD although the pathological process involving the PPN seems different between these conditions(Galazky *et al.*, 2019) and this may be consistent with the different type of sleep (but also gait) alterations observed in PSP compared to PD(Mantovani *et al.*, 2018). In particular, excessive daytime sleepiness is considered a frequent non-motor symptom in PD(Schapira *et al.*, 2017) while, as previously discussed, it is uncommon in PSP. In PD, excessive daytime sleepiness may also be exacerbated by dopaminergic medications, although some studies do not support this hypothesis(Chahine *et al.*, 2017).

Limitations of this study include the small number of patients, however considering the complexity of the tests performed in this study and the rarity of this condition this sample is relatively considerable. Two patients were not able to complete the recording and more patients did not tolerate the oronasal sensor which they removed overnight. The diagnoses did not have neuropathological confirmation, however all patients had Richardson's syndrome but one that had parkinsonian variant. These are the most specific for PSP pathology. In addition, none of the patients had red flags for other conditions and all had brain MRI with spectroscopy suggesting PSP. Therefore the diagnosis of PSP was made with high level of probability. Finally we did not have controls to compare to our patients.
Future directions of this study include the possibility to compare PSP patients with other patients that performed the same protocol (MSA and PD patients) and age ad sex-matched controls to better elucidate which particular aspects may serve as discriminant and characterizing element for one condition over another.

Section II

BODY CORE TEMPERATURE CIRCADIAN RHYHTM AND STATE-DEPENDENT MODULATION IN PROGRESSIVE SUPRANUCLEAR PALSY

BACKGROUND

Circadian rhythms were introduced in the previous section regarding the sleep-wake cycle. It is noteworthy that virtually all physiological functions of the human body are regulated by a biological clock that sets a rhythm to alternate a period of activity to a period of repose. This rhythm mainly follows the light-dark cycle and is of approximately 24 hours (*circadian*). Disruption of circadian rhythms has proven to have detrimental effects on human health and plays a major role in the development of cardiovascular, metabolic and indeed neurodegenerative diseases(Videnovic *et al.*, 2014). The central clock is the suprachiasmatic nucleus (SCN) located in the anterior hypothalamus(Hastings *et al.*, 2018). Additional "peripheral" clocks are found in tissues and organs and mediate the effect of the central clock on the target cells. The SCN integrates external and internal stimuli and exerts its output function by means of neuronal pathways (the autonomic nervous system) and hormones (melatonin, cortisol).

Few studies have investigated this fascinating topic in progressive supranuclear palsy (PSP).

Body core temperature rhythm and state-dependent modulation

Body core temperature (BcT) is regulated by a homeostatic process controlled in the preoptic/anterior hypothalamic area to maintain BcT values within normal physiological range. BcT is also regulated by the circadian system (SCN of the hypothalamus), presenting a 24 h variability with maximal values in the late afternoon and minimal in the early morning. Assessing BcT circadian rhythm in controlled environmental conditions (to avoid external confounding factors) provides a strong indicator of circadian system integrity and autonomic nervous system function (the sympathetic nervous system is one of the main thermoregulation effectors being responsible for heat conservation and metabolic thermogenesis through control of vasomotor tone and involuntary shivering and heat loss through control of the sudomotor system(Collins, 2013)).

Being both under the influence of the SCN, thermoregulation and sleep are closely connected. On the one hand, humans usually initiate sleep on the downward slope of the circadian BcT rhythm, when the rate of decrease of BcT and peripheral heat loss via selective vasodilatation in distal skin regions are maximal and when endogenous levels of melatonin begin to rise. Such body heat loss promotes the ability to initiate and maintain sleep(Krauchi and Deboer, 2010). On the other had, the different sleep (NREM and REM) and wake states themselves influence thermoregulation. As a matter of fact, BcT is set at a lower level during NREM sleep than during wake, as an energy conservation function typical of sleep. During NREM sleep, thermoregulatory responses are operative, while REM sleep is characterized by a marked inhibition of thermoregulation, with changes in body temperature occurring passively in relation to the environmental heat load(Krueger and Takahashi, 1997; Szymusiak, 2018).

So far, only one study assessed BcT circadian rhythm in PSP. In 2009, Suzuki and colleagues recorded rectal temperature in controlled environmental conditions for 48 consecutive hours in 5 PSP and 16 PD

patients(Suzuki *et al.*, 2009). The results showed that BcT rhythm was preserved in all patients, and no significant differences where found among groups regarding mesor and acrophase. However, BcT amplitude was lower in PSP than PD and mean BcT was significantly higher from 23:00 to 2:00 in PSP than PD. The Authors conclude that different brainstem neurodegenerative patterns between PD and PSP may play a role, particularly an earlier and more severe involvement of brainstem autonomic nuclei and pedunculopontine nucleus in PSP. This study was limited by the small number of PSP patients and the lack of concomitant sleep study to evaluate the role of sleep in BcT regulation.

Neuropathology of the circadian system

Key central structures of the circadian system (the SCN and the pineal gland, the site of production of melatonin) were analysed in one neuropathological study(Pablo-Fernández *et al.*, 2018). In PSP, the SCN showed PSP-related tau pathology of mild to moderate severity in all cases, but no tau pathology was found in pineal tissue. Therefore this study demonstrated the involvement of certain key structures of the circadian system (SCN but not pineal gland) in PSP.

In summary, clinical and neuropathological studies demonstrated that circadian rhythms are impaired in PSP. Certain functions seem more compromised than others (sleep-wake cycle over BcT), and it should be noticed that this topic was not addressed in many studies and those available were performed on a small number of patients. Nonetheless, circadian rhythm disruption results in clinical complaints and poor quality of life which, in turn, lead to additional burden for patients and caregivers. Moreover, given the role that circadian rhythm disruption plays in neurodegeneration, targeting this topic to better elucidate the pathomechanisms and possible treatment may improve not only patients' symptoms and quality of life but possibly prognosis as well.

The aim of this study was to examine the circadian rhythm of BcT in PSP patients under controlled environmental conditions and the interactions between sleep and BcT by comparing BcT values across sleep (NREM and REM) and wake states (state-dependent modulation).

MATERIALS AND METHODS

Participants

Thirteen patients with PSP diagnosed according to consensus criteria were enrolled(Höglinger *et al.*, 2017). Two patients were not able to complete study protocol because during the first night removed most of the recording set. One further patient refused the BcT recording. Therefore 10 patients completed the study protocol. None of the patients had concomitant severe medical conditions that could have affected study results. All patients gave their written informed consent to participate in the study. A group of 7 healthy

male controls that performed the same protocol at the same Institution were used for comparison. All healthy individuals gave their written informed consent to personal data processing for research purposes.

Study protocol

All patients were admitted to the hospital to complete the study protocol. Upon admittance, all subjects underwent general and neurological clinical examination. In the afternoon they received an enema to prepare for rectal temperature monitoring. The patients were catheterized for the whole duration of the protocol. On the following morning, sleep-wake cycle, body core temperature (BcT), arterial blood pressure (BP) and heart rate (HR) were continuously monitored for 48 hours under controlled environmental conditions and according to standardized procedures.

Sleep-wake cycle was monitored by an ambulatory polygraphic recorder (Albert Grass Heritage®, Colleague TM PSG Model PSG16P-1, Astro-Med, Inc, West Warwick, RI, USA or Neurofax Electroencephalograph EEG-1200, Nihon-Kohden Corp., Tokyo, Japan). Polygraphic recording included electroencephalogram (EEG: Fp_1-F_3, F_3-C_3, C_3-P_3, P_3-O_1, Fp_2-F_4, F_4-C_4, C_4-P_4, P_4-O_2, C_z-P_z), right and left electrooculogram (EOG), electrocardiogram (EKG), electromyogram (EMG) of the mylohyoideus, intercostalis, extensor carpi (left and right) and anterior tibialis (left and right) muscles, thoraco-abdominal breathing (strain gauge) and video-synchronized recording. Oronasal airflow sensor and pulse oximetry were applied after the subject finished dinner and maintained for the night-time.

Rectal temperature was monitored every 2 minutes by a Mini-logger™ (Bend, Oregon, USA) portable device.

During the study, subjects were allowed to sleep ad libitum, living in a temperature (24 ± 1 °C) and humidity (40-50%) controlled room, lying in bed except when eating, in a light-dark schedule (dark period: 23:00 - 7:00). The subjects were placed on a 1.800 kcal/day diet, divided into three meals (8:00, 12:00, 18:00) and three snacks (10:00, 16:00, 23:00). From midnight preceding the monitoring, subjects were instructed to avoid alcohol and caffeinated beverages and to abstain from smoking.

Body core temperature parameters

Day-night pattern of BcT

To evaluate day-night pattern of BcT, daytime (from 7:00 to 23:00) and night-time (from 23:00 to 7:00) mean values were calculated. Nocturnal BcT decline was determined by the difference between night-time and daytime values (ΔBcT).

24-hr circadian rhythm of BcT

Rhythmicity was analysed by evaluating the time series for BcT according to the single cosinor method, using a computerized procedure (Chronolab) (Mojón *et al.*, 1992). In brief, this procedure elaborates the raw temperature curve obtained across the 24-hour period using a specific mathematical curve-fitting

method (cosine curve). The procedure then determines whether or not there is a rhythm with a 24-hour period and evaluates the following parameters within their 95% confidence limits: (1) mesor (Midline Estimating Statistic of Rhythm), that is the mean value of the cosine function; (2) amplitude, defined as the difference between the maximum value measured at the acrophase and the mesor of the cosine curve used to approximate the rhythm; and (3) acrophase, defined as the interval between midnight hour (reference time) and time of highest value of the cosine function (**Figure 1**). The acrophase indicates the presence of circadian rhythmicity and the timing of peak of activity (phase), hence exploring the function of the suprachiasmatic nucleus of the hypothalamus (SCN). Conversely, mesor and amplitude are representative of the homeostatic thermoregulatory process regulated by the preoptic/anterior area of the hypothalamus (POA). For each patient, the 24-h rhythmicity of the last 24 hours of recording is reported.

State-dependent analysis of BcT

The mean value of BcT in wake and each sleep stage (NREM stage 1, 2, 3 and REM sleep) was determined over the 24-hr period. The difference (Δ) between the mean value of BcT in every sleep stage during the dark period and the mean value in wake, considered as reference value, was also calculated.

Statistical analysis

Normality was checked for each variable with the Shapiro-Wilk test. Comparisons between patients and controls were performed with Student *t*-test for normally distributed data and Mann-Whitney test for not normally distributed data. Categorical variables were compared with the chi-square test. Statistical significance was set at $p \leq 0.05$. Data were analysed using SPSS Statistics (version 25).

RESULTS

Demographics and clinical characteristics of the subjects are reported in **Table 1**. PSP patients were significantly older than controls and gender was not equally distributed between the two groups ($p > 0.05$). BcT parameters are reported in **Table 2** and **Figure 2**. All PSP patients presented the expected nocturnal decline in BcT (that is, BcT night < BcT day). PSP patients presented significantly higher BcT values during day and night compared to controls, however the BcT Δ was comparable.

A circadian rhythmicity of BcT was detected in all PSP patients. Mesor was significantly higher in PSP than controls, whereas amplitude and acrophase were similar.

The mean values of BcT during wake, NREM 1, NREM 2 and REM were significantly higher in PSP than controls. However, there were no significant differences between PSP and controls when comparing the BcT Δ in each sleep stage with respect to wake. State-dependent analysis is shown in **Figure 3**.

Table 1_Demographics and clinical characteristics of PSP patients and controls

	PSP	Controls
N	10	7
Age (years)	74.5 ± 2.8*	45.6 ± 10.9
Males (n, %)	3 (30%)	7 (100%)
BMI (Kg/m^2)	28.5 ± 4.0	29.0 ± 4.0
Disease duration (years)	4.9 ± 1.7	n.a.
Hohen &Yahr stage	3.6 ± 0.7	n.a.
PSPRS	46.7 ± 9.1	n.a.
MMSE	25.2 ± 2.2	n.a.

* $p \leq 0.05$

Legend: PSP = progressive supranuclear palsy; BMI = body mass index; PSPRS = PSP rating scale; MMSE = mini mental state examination.

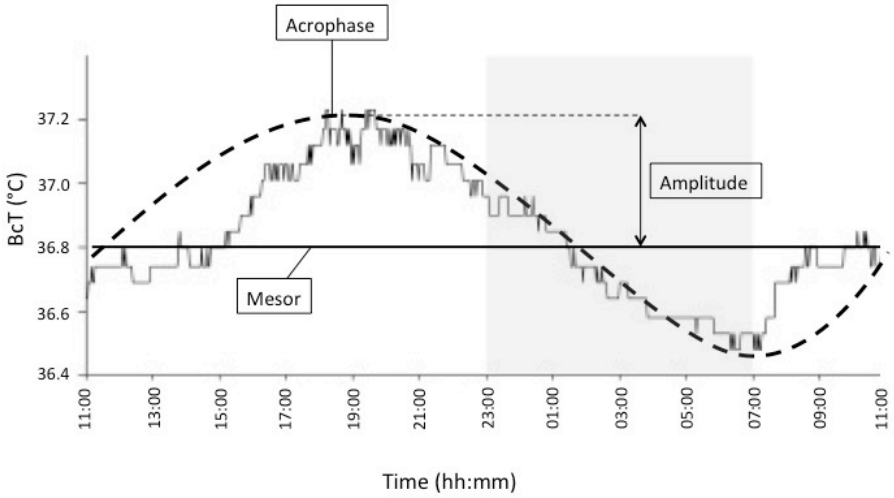
Table 2_Body core temperature parameters in PSP patients and controls

	PSP	Controls
BcT day (°C)	37.34 ± 0.20*	37.08 ± 0.17
BcT night (°C)	37.06 ± 0.33*	36.68 ± 0.19
BcT Δ (°C)	-0.28 ± 0.26	-0.40 ± 0.18
Mesor (°C)	37.22 ± 0.21*	36.97 ± 0.15
Amplitude (°C)	0.28 ± 0.16	0.35 ± 0.15
Acrophase (°)	269.8 ± 47.2	253.7 ± 15.3
Wake_BcT (°C)	37.30 ± 0.19*	37.01 ± 0.16
N1_BcT (°C)	37.03 ± 0.31*	36.73 ± 0.15
N2_BcT (°C)	37.05 ± 0.33*	36.67 ± 0.23
N3_BcT (°C)	37.01 ± 0.32	36.71 ± 0.19
R_BcT (°C)	37.00 ± 0.37*	36.61 ± 0.23
N1_Δ (°C)	-0.27 ± 0.23	-0.28 ± 0.17
N2_ Δ (°C)	-0.25 ± 0.27	-0.34 ± 0.23
N3_ Δ (°C)	-0.31 ± 0.21	-0.30 ± 0.16
R_ Δ (°C)	-0.30 ± 0.25	-0.40 ± 0.20

* $p \leq 0.05$

Legend: PSP = progressive supranuclear palsy; BcT = body core temperature; BcT Δ = BcT night - BcT day; N1 = NREM sleep stage 1; N2 = NREM sleep stage 2; N3 = NREM sleep stage 3; R = REM sleep; N1_ Δ = BcT N1 - BcT wake; N2_ Δ = BcT N2 - BcT wake; N3_ Δ = BcT N3 - BcT wake; R_ Δ = BcT REM - BcT wake.

Figure 1_Parameters of circadian rhythm analysis



Visual representation of a normal 24-h profile of BcT and parameters of the circadian rhythm analysis. Shaded area indicates night-time. Legend: BcT = body core temperature

Figure 2_ Body core temperature parameters in PSP and controls

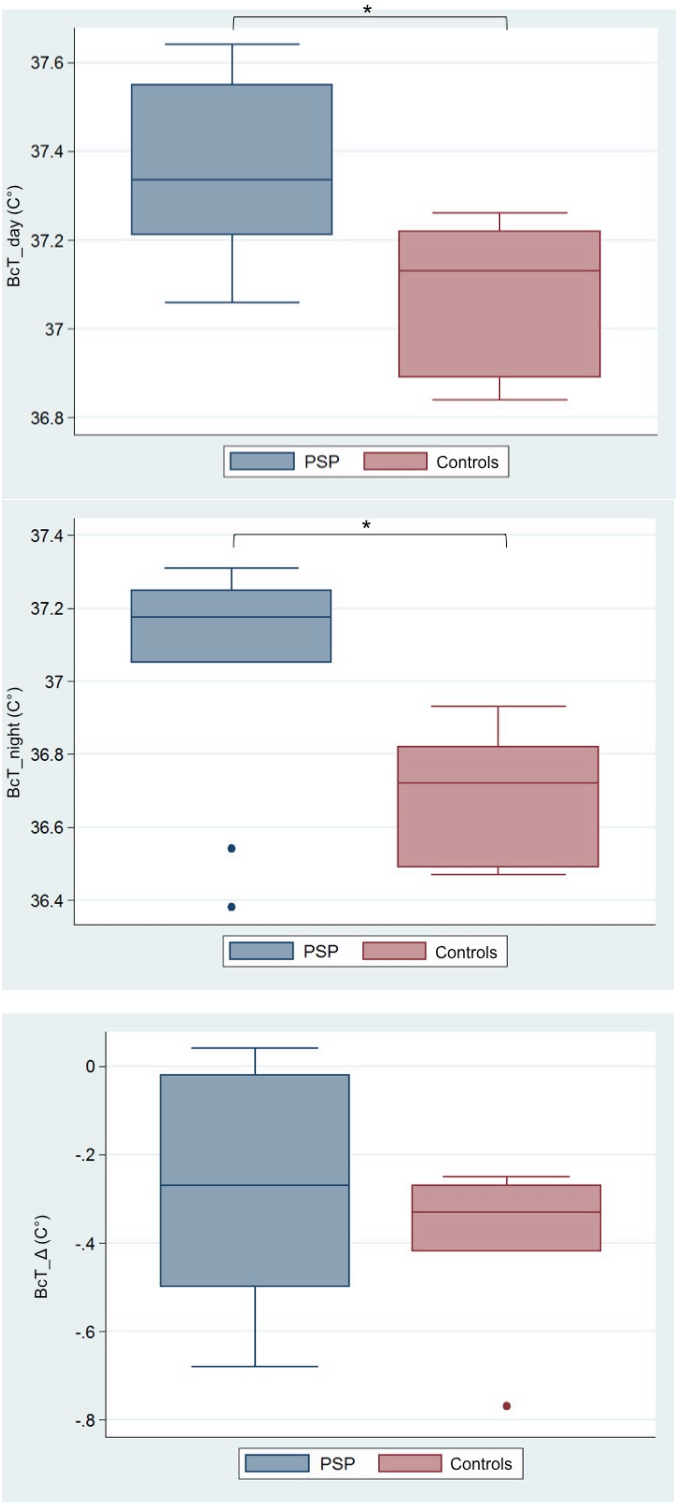
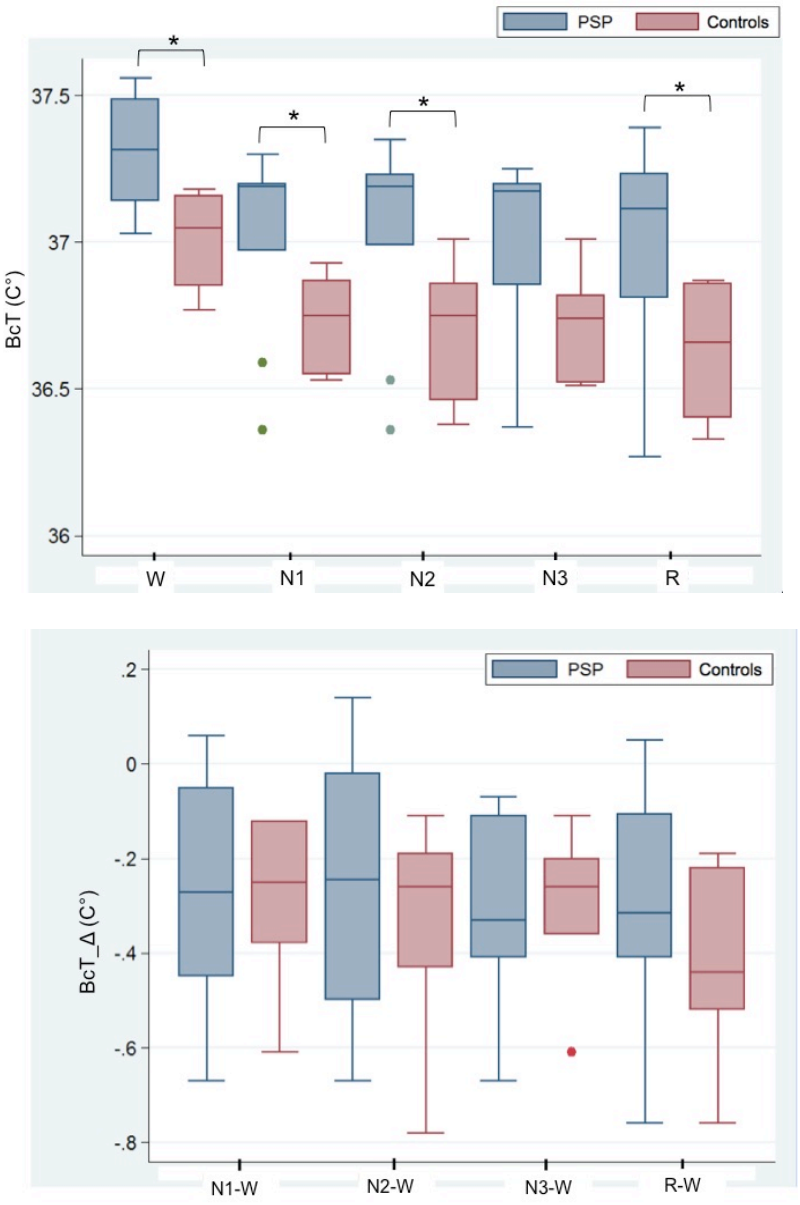


Figure 3_State-dependent analysis of body core temperature in PSP and controls



Legend: PSP = progressive supranuclear palsy; BcT = body core temperature; W = wake; N1 = NREM sleep stage 1; N2 = NREM sleep stage 2; N3 = NREM sleep stage 3; R = REM sleep; Δ = difference (BcT sleep stage – BcT wake).

DISCUSSION

This is the first study to investigate the circadian rhythm and state-dependent modulation of BcT in a cohort of PSP patients under controlled environmental conditions. PSP presented the physiological night-time decrease of BcT, showing a normal pattern across the 24 hours. Comparing PSP results to those of the control group, PSP had significantly higher mean day and night values, but similar BcT Δ. Therefore the expected variability of BcT (night-time BcT fall) is present in PSP as it is in normal subjects. Regarding the circadian rhythm study, all PSP patients presented BcT rhythmicity. Compared to controls, PSP had similar acrophase and amplitude, whereas mesor was significantly higher. These data seem to suggest that PSP have preserved circadian rhythmicity (expressed by the acrophase) but may have an impairment of the homeostatic control (i.e. increased mesor) of thermoregulation. Further new insights came from the state-dependent analysis. PSP presented higher mean values of BcT during wake, NREM 1, NREM 2 and REM sleep, however there were no significant differences in the Δ (mean BcT value in each sleep stage – BcT wake). This suggests that PSP maintain the physiological state-dependent modulation of BcT between wake, NREM and REM sleep. The higher mean BcT values observed may reflect an impairment of sympathetic function, particularly an inability to reduce sympathetic function during sleep or they may be a consequence of the altered sleep structure (higher representation of light sleep) demonstrated by the PSP patients as discussed in the previous section.

This study confirm the results of the only former study assessing BcT circadian rhythm in PSP, which found a normal circadian function in all patients(Suzuki *et al.*, 2009) although a lower amplitude compared to PD. Pierangeli et al. in 2001 performed a study on BcT circadian rhythm and state-dependent modulation in 7 patients with PD, 14 MSA and 8 controls(Pierangeli *et al.*, 2001). The Authors found that PD and controls showed similar BcT mesor, amplitude and acrophase, as well as similar BcT values in NREM and REM sleep, whereas MSA had significantly higher mesor and BcT during sleep stages NREM 1-2, NREM 3-4 and REM compared to both PD and controls. As in the present study, the controls were significantly younger however these results were maintained also when comparing MSA against the pooled PD-control groups, suggesting that age does not play a significant confounding factor. Interestingly, PSP patients in our study showed a similar BcT profile to the MSA patients of Pierangeli et al.

As mentioned in the introduction, the circadian rhythm of BcT also provides information on central autonomic function and on the sympathetic efferents that induce thermoregulatory vasomotion and sweating. Several areas of the Central Autonomic Network and preganglionic sympathetic neurons are known to be affected in MSA, whereas the degree of involvement of the autonomic nervous system in PSP is less clear. Very few neuropathological studies investigated autonomic structures in PSP. One study demonstrated consistent abnormalities in several autonomic nuclei located in the brainstem (medial and parabrachial nuclei, gigantocellular reticular nucleus, raphes magnus and raphes obscurus nuclei,

intermediate reticular zone) which are mainly related to cardiovascular function(Rüb *et al.*, 2002). The intermediolateral column in the spinal cord was found to be spared(Iwasaki *et al.*, 2007) or moderately affected (39% nerve cell loss) by the neurodegenerative process in PSP(Vitaliani *et al.*, 2002).
Alternatively, the alterations of the thermoregulatory homeostatic control observed in PSP may reflect the sleep/wake cycle disturbances discussed in the previous section. A disruption of the homeostatic sleep drive as been suggested as PSP patients tend to show profound sleep loss during the night without compensatory napping during the day, i.e. an hyperarousal state. This may in turn alter the appropriate heat exchanges, particularly it may favour an inability to reduce sympathetic activity to produce the peripheral vasodilatation necessary for heat loss and BcT fall, thus maintaining mean BcT values above the expected range.
Even though tau pathology was described in the SCN of PSP patients(Pablo-Fernández *et al.*, 2018), circadian rhythmicity was maintained therefore such finding does not seem to have a clinical correlate regarding BcT circadian rhythm. Ideally, having clinical and neuropathological data from the same individuals would provide stronger evidence in this regard. The role of melatonin should also be further investigated. The same neuropathological study found preserved pineal gland tissue however no study so far investigated melatonin profile and its association to sleep and BcT parameters in PSP patients. This would add important information and may also be useful from a therapeutic point of view as melatonin supplementation is available.

Limitations of this study is the small number of patients (further restricted by the fact that some patients were not able to complete the protocol for episodes of nocturnal confusion in the hospital setting) and the lack of pathological confirmation, however all patients had Richardson's phenotype but one patient that had the parkinsonian variant and none had red flags suggesting other diagnoses. Our controls were younger and we did not have available data from other patients' groups to compare directly to our PSP. Future directions of this study include collecting and comparing data from patients with other neurodegenerative diseases like PD and MSA which may provide interesting new information on this topic.

Section III

CARDIOVASCULAR AUTONOMIC FUNCTION IN PROGRESSIVE SUPRANUCLEAR PALSY

Autonomic control of the cardiovascular system during wakefulness

BACKGROUND

Orthostatic hypotension (OH) is defined as a sustained reduction of at least 20 mmHg of systolic blood pressure (SBP) and/or 10 mmHg of diastolic blood pressure (DBP) within 3 minutes of standing or head-up tilt test (HUTT)(Freeman *et al.*, 2011). OH may be secondary to other factors such as medications (especially anti-hypertensive and dopaminergic drugs), dehydration, concomitant cardiac disease, among others, or it can be a manifestation of cardiovascular autonomic failure (i.e. neurogenic OH, NOH). When OH occurs beyond 3 minutes of orthostatic stress, it is called delayed OH, and is considered a possible marker of initial sympathetic failure(Gibbons and Freeman, 2006). OH may manifest with symptoms of orthostatic intolerance (e.g. blurring of vision, light-headedness) culminating in syncope, or it can be totally asymptomatic. The neurogenic nature of OH can be identified by performing autonomic testing, especially the Valsalva manoeuvre (VM). The absence of the increase in blood pressure above baseline values at the end of the VM (overshoot) indicates sympathetic neurocirculatory failure and, if associated with OH, marks it as neurogenic(Jain and Goldstein, 2012; Cheshire *et al.*, 2021). About half the patients with NOH presents supine hypertension (SH), defined by a SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg after at least 5 min of supine rest(Fanciulli *et al.*, 2018). This condition, properly named neurogenic SH, also represents a marker of cardiovascular dysautonomia and might be particularly severe at night-time when the subject maintains the supine position for several hours (nocturnal hypertension).

OH prevalence in progressive supranuclear palsy (PSP) ranged from 0 to 45%(Wenning *et al.*, 1999; Kimber *et al.*, 2000; Kikkawa *et al.*, 2003; Schmidt *et al.*, 2008; Bae *et al.*, 2009; Schmidt, Berg, *et al.*, 2009; Schmidt, Herting, *et al.*, 2009; Reimann *et al.*, 2010; Oliveira *et al.*, 2019; Van Gerpen *et al.*, 2019). The large variability across studies could be ascribed to different study designs (cross-sectional or retrospective) and methods of assessments (HUTT or standing or both). Among cross-sectional clinical studies, 2 did not find OH in any PSP patient(Kimber *et al.*, 2000; Kikkawa *et al.*, 2003), whereas the majority found a prevalence between 8 and 33%(Schmidt *et al.*, 2008; Bae *et al.*, 2009; Schmidt, Berg, *et al.*, 2009; Schmidt, Herting, *et al.*, 2009; Reimann *et al.*, 2010). However, 4 out of 5 of those who found some degree of OH were conducted on the same cohort of PSP(Schmidt *et al.*, 2008; Schmidt, Berg, *et al.*, 2009; Schmidt, Herting, *et al.*, 2009; Reimann *et al.*, 2010). Retrospective studies with pathological confirmation also provided conflicting results. Van Gerpen et al. did not find OH in 14 PSP previously assessed with formal autonomic testing(Van Gerpen *et al.*, 2019). Oliveira and colleagues found instead a 9% prevalence of documented or symptomatic OH in their large cohort of PSP (104 patients, the study with the largest numerosity)(Oliveira *et al.*, 2019). Conversely, Wenning and co-workers reported the highest prevalence (45%) of OH tested with standing at bedside in 24 patients(Wenning *et al.*, 1999).

The potential, confounding, hypotensive role of dopaminergic medication should be taken into account when interpreting these results. In 3 studies, cardiovascular autonomic tests were performed after a washout(Kimber *et al.*, 2000; Reimann *et al.*, 2010; Van Gerpen *et al.*, 2019). In the remaining, these

medications were not discontinued prior to testing(Schmidt *et al.*, 2008; Schmidt, Berg, *et al.*, 2009; Schmidt, Herting, *et al.*, 2009) or it was not specified(Wenning *et al.*, 1999; Kikkawa *et al.*, 2003; Bae *et al.*, 2009; Oliveira *et al.*, 2019). It is noteworthy that 2 out of 3 studies where dopaminergic drugs were discontinued prior to HUTT found no OH. This may imply that the higher prevalence found in other studies may at least partly reflect OH secondary to medications.

Some studies highlighted the discrepancy between the number of patients with OH according to criteria and those who were also symptomatic, the latter being usually a minority. For example, Schmidt et al. found that while 16% had OH, only 6% were symptomatic during HUTT(Schmidt *et al.*, 2008). Similarly, Bae and collaborators found no correlation between complaints of orthostatic intolerance and the presence of OH(Bae *et al.*, 2009).

A similar median latency for OH (30 months(Wenning *et al.*, 1999) and 2 years(Oliveira *et al.*, 2019)) was reported by some retrospective studies with regular follow-up.

NOH was specifically addressed only by Van Gerpen and colleagues(Van Gerpen *et al.*, 2019), who reviewed medical charts and autonomic testing of 14 autopsy-confirmed PSP patients. NOH, defined by a SBP drop of at least -30 mmHg at 5 min of HUTT associated with a pathological VM or a blunted reflex tachycardia ($\Delta HR/\Delta SBP$ ratio < 0.5) or CASS adrenergic subscore > 2, was not found in PSP.

Cardiovascular sympathetic and parasympathetic functions were further investigated by means of autonomic testing. Sympathetic function was mainly derived from the entity of BP drop on orthostatic test and BP responses to VM and isometric exercise, whereas parasympathetic activity was based on HR variability during the VM, deep breathing or orthostatic test. While an overt autonomic failure was never found, some studies concluded for a certain degree of autonomic impairment involving both branches(Sandroni *et al.*, 1991; van Dijk *et al.*, 1991; Gutrecht, 1992; Friedrich *et al.*, 2008, 2010; Schmidt *et al.*, 2008; Schmidt, Berg, *et al.*, 2009; Schmidt, Herting, *et al.*, 2009; Reimann *et al.*, 2010; Nojszewska *et al.*, 2019) in contrast to others that found substantial intact responses(Brefel-Courbon *et al.*, 2000; Kimber *et al.*, 2000; Holmberg *et al.*, 2001; Deguchi *et al.*, 2002; Kikkawa *et al.*, 2003). Van Gerpen et al deduced that adrenergic system is relatively preserved in PSP, whereas indices of cardiovagal function could be abnormal similarly to other parkinsonian syndromes (MSA, DLB)(Van Gerpen *et al.*, 2019).

Most research compared PSP not only to controls but also to other parkinsonian disorders, particularly PD and MSA. While MSA showed a more severe autonomic failure in all studies, the comparison between PSP and PD gave conflicting results.

Orthostatic intolerance symptoms investigated by means of questionnaires (Non-Motor Symptom Scale, Autonomic Symptom Questionnaire) were reported by 20-50% of PSP patients(Ou *et al.*, 2016; Radicati *et al.*, 2017; Nojszewska *et al.*, 2019; Chaithra *et al.*, 2020) and by 13% on structured interview(Colosimo *et al.*, 2010). One study reported a mean score of 12 for the cardiovascular domain on the SCOPA-Aut questionnaire, which was intermediate between the score of MSA (the highest) and PD (lowest)(Berganzo

et al., 2012). These studies did not have an objective confirmation of OH, nevertheless the general lower prevalence of OH reported by studies mentioned above seems to suggest that the questionnaires may overestimate OH. Indeed, Bae et al. found that orthostatic symptoms at the Autonomic Dysfunction Questionnaire were complained by 42% of PSP patients but only 33% actually presented OH and, as already discussed previously, no correlation was found between symptoms and OH objective presence(Bae *et al.*, 2009). Likewise, 63% of PSP patients reported cardiovascular symptoms during a standardized clinical interview, yet autonomic testing disclosed OH only in 16% of cases(Schmidt *et al.*, 2008).

The aim of this study was to objectively evaluate cardiovascular autonomic function and the presence of NOH (in contrast to secondary OH) by means of cardiovascular reflex tests (CRTs) performed according to standardized procedures in a cohort of patients with PSP, and to compare results with other parkinsonian syndromes (PD, MSA) to possibly identify differentiating patterns.

MATERIALS AND METHODS

Participants

Thirty-five patients with PSP diagnosed according to consensus criteria(Höglinger *et al.*, 2017) (recruited retrospectively or prospectively) were included. For comparisons were considered a group of sex- and age-matched controls and two groups of PD(Postuma *et al.*, 2015) and MSA(Gilman *et al.*, 2008) patients with similar disease duration to PSP. None of the subjects had symptoms or signs of poorly compensated diabetes, cardiorespiratory disease or other pathological conditions that might have affected autonomic cardiovascular control. All subjects had given written informed consent to personal data processing for research purposes.

Cardiovascular reflex tests

All CRTs were performed at our Institution according to standardized procedures (Corazza *et al.*, 2014; Baschieri *et al.*, 2015). CRTs were performed in the morning, in a temperature-controlled clinical investigation room (23 ± 1 °C). Subjects had been drug-free overnight and were allowed to have only a light breakfast in the morning avoiding coffee and tea and refraining from smoke. CRTs were performed under audio and video-polygraphic monitoring (ANScovery Modular System, SparkBio Srl, Bologna, Italy). During the tests the following parameters were monitored continuously: beat to beat BP (Finometer Midi, Finapres Medical Systems, Amsterdam, The Netherlands), EKG, oronasal and abdominal breathing and peripheral vasomotor tone (Model 15LT, Grass Techonologies, Quincy, MA). All parameters were acquired and sampled at a rate of 500Hz. The ANScovery software was used to visualize, store and analyse the data, providing a final report with the results.

After 30 min of supine rest, the following tests were performed: HUTT (10 min at 65°), VM (forced expiratory pressure of 40 mmHg maintained for 15 seconds), deep breathing (DB, 6 breaths/min), cold face

(CF, cold stimulus on forehead for 1 minute) and sustained handgrip tests (HG, 1/3 of maximal effort for 5 min). An adequate period of rest was allowed to reach basal BP and HR values in-between investigations. A specialized technician and an external device tutor monitor guided and supported subjects during the execution of CRTs. The correct execution of each test was checked automatically by an electronic device and by a specialized technician.

This integrated instrumental method of autonomic evaluation automatically calculated the following parameters: 1) basal SBP, DBP and HR as the mean value of the last 5 min of supine rest preceding HUTT; 2) response to HUTT as the difference (Δ) between SBP, DBP and HR values at 3° min and basal ones; 3) Valsalva ratio (VR) = HR in phase II/HR in phase IV of the VM; 4) presence of BP recovery in late phase II of the VM (Δ BP IIb-IIa) = max BP in late phase II - min BP in early phase II; 5) presence of overshoot in phase IV of the VM = max BP phase IV (within 20 seconds after the strain release) – mean basal BP; 6) sinus arrhythmia during DB (ΔIE) = average of the 10 shortest R-R intervals during inspiration and average of the 10 longest R-R during expiration; 7) response to CF as Δ compared to basal values of SBP, DBP and HR after 1 min of cold stimulus on the forehead; 8) response to HG as Δ compared to basal values of SBP, DBP and HR after 5 min of isometric effort.

Statistical analysis

Comparisons between groups were performed with an analysis of covariance model with age, sex and BMI as confounding factors. A multiple linear regression model was employed to explore the relations of disease duration, H&Y and MMSE on variables of interest in the PSP group. Finally, a discriminant analysis was performed between the PSP and PD group. In particular, we tried to predict the group of an observation using a simple but powerful approach to prediction, i.e., classification trees. Unlike other approaches to classification/prediction, trees do not define a prediction equation. Instead, data are partitioned along the predictor axes into subsets with homogeneous values of the dependent variable(Breiman *et al.*, 1984). The resulting process is well depicted by a tree that can be used to make predictions from new observations. An important added value of classification trees is that they automatically select the most relevant discriminant variables that should be used to take a decision on the most likely group for an observation. These variables can subsequently be used to formulate clinical hypotheses and inform future research. All analyses were performed using the software R(R Core Team, 2017). Statistical significance was set at $p \leq 0.05$.

RESULTS

Demographics and clinical characteristics of the participants are shown in **Table 1**. At the time of autonomic testing, there were no significant differences in disease duration between patients' groups (PSP: 5.5 ± 3.0 years; PD: 5.5 ± 2.8 years; MSA: 5.5 ± 2.3 years). Similarly, gender and BMI were comparable among

patients' groups as well as with healthy controls. Age at autonomic testing was similar between PSP (73.0 ± 4.9 years) and healthy controls (72.7 ± 4.4 years). Conversely, compared to PD (69.0 ± 6.1 years) and MSA (66 ± 6), PSP resulted significantly older. Disease severity expressed as Hoehn & Yahr stage was higher in PSP than PD. More than half of the patients were being treated with levodopa or levodopa + dopamine agonist. LEDD was comparable between all groups. The percentage of PSP patients treated with anti-hypertensive drugs (83%) was significantly higher than other groups (PD: 43%; MSA: 13%; healthy controls: 53%). The rest of concomitant medications resulted comparable.

Results of CRTs are shown in **Table 2** and **Figure 1**.
OH was detected in 5/35 (14%) PSP, 3/40 (8%) PD and 25/40 (63%) MSA. However, no PSP patient presented NOH (associated with a pathological response to the VM, particularly absence of overshoot). Instead, OH was neurogenic in all PD patients and in 24 MSA patients. Finally, OH was found in one subject of the control group (non-neurogenic OH).

At supine rest, SBP was significantly higher in PSP than PD and controls. In addition, PSP had higher DBP compared to controls and higher HR compared to PD.
Compared to healthy controls, PSP presented significant ($p < 0.05$) differences in all the responses to the VM, handgrip test and SBP response to cold face test. Such differences were markedly significant ($p < 0.0001$) for handgrip test parameters.
Compared to PD, PSP showed significantly ($p < 0.05$) lower cardiovagal response to deep breathing, lower DBP and HR response to handgrip test, and higher BP responses to cold face test.
Patients with MSA presented significantly ($p < 0.05$) more compromised results to all tests (with the exception of HR response to handgrip test, $p = 0.1$) compared to PSP.
Regardless of significant differences obtained from comparisons between groups, it is noticeable that responses to CRTs in the PSP group were within normal range.

Looking at the potential effect of clinical variables on CRTs results, disease duration significantly influenced SBP response at HUTT ($p = 0.03$) and there was a trend toward significance for rest supine DBP ($p = 0.07$) and rest supine HR ($p = 0.08$). Hoehn & Yahr stage had a significant effect on VR ($p = 0.01$).

Results of the discriminant analysis performed between PSP and PD are reported in **Figure 2**. The parameters that better differentiated PSP from PD were supine rest SBP, DBP response to cold face test and HR response to handgrip test.

Table 1_Demographics and clinical characteristics

	PSP	PD	MSA	Controls
N	35	40	40	34
Age (years)	73.0 ± 4.9	69.0 ± 6.1*	66 ± 6*	72.7 ± 4.4
Males (n, %)	18 (51%)	28 (70%)	26 (65%)	18 (53%)
BMI (Kg/m^2)	28.0 ± 3.6	27.4 ± 3.7	26.8 ± 3.5	27.7 ± 4.9
Disease duration (years)	5.5 ± 3.0	5.5 ± 2.8	5.5 ± 2.3	n.a.
PSPRS	45.8 ± 13.3	n.a.	n.a.	n.a.
H&Y	3.6 ± 0.7	2.0 ± 0.6	3.3 ± 1.2	n.a.
Dopaminergic therapy				n.a.
Levodopa	19 (54%)	14 (35%)	15 (38%)	
Dopamine agonist	0	0	0	
Levodopa + dopamine agonist	6 (17%)	25 (63%)	4 (10%)	
LEDD (mg)[a]	450 (100-1000)	380 (100-1315)	400 (50-1401)	
Other therapy				
Hypertension	29 (83%)	17 (43%)*	5 (13%)*	18 (53%)
Diabetes	5 (14%)	2 (5%)	0	3 (9%)
Prostate hyperplasia (α-blockers)	4 (11%)	4 (10%)	2 (5%)	2 (6%)
Depression	12 (34%)	5 (12.5%)*	7 (18%)	4 (12%)

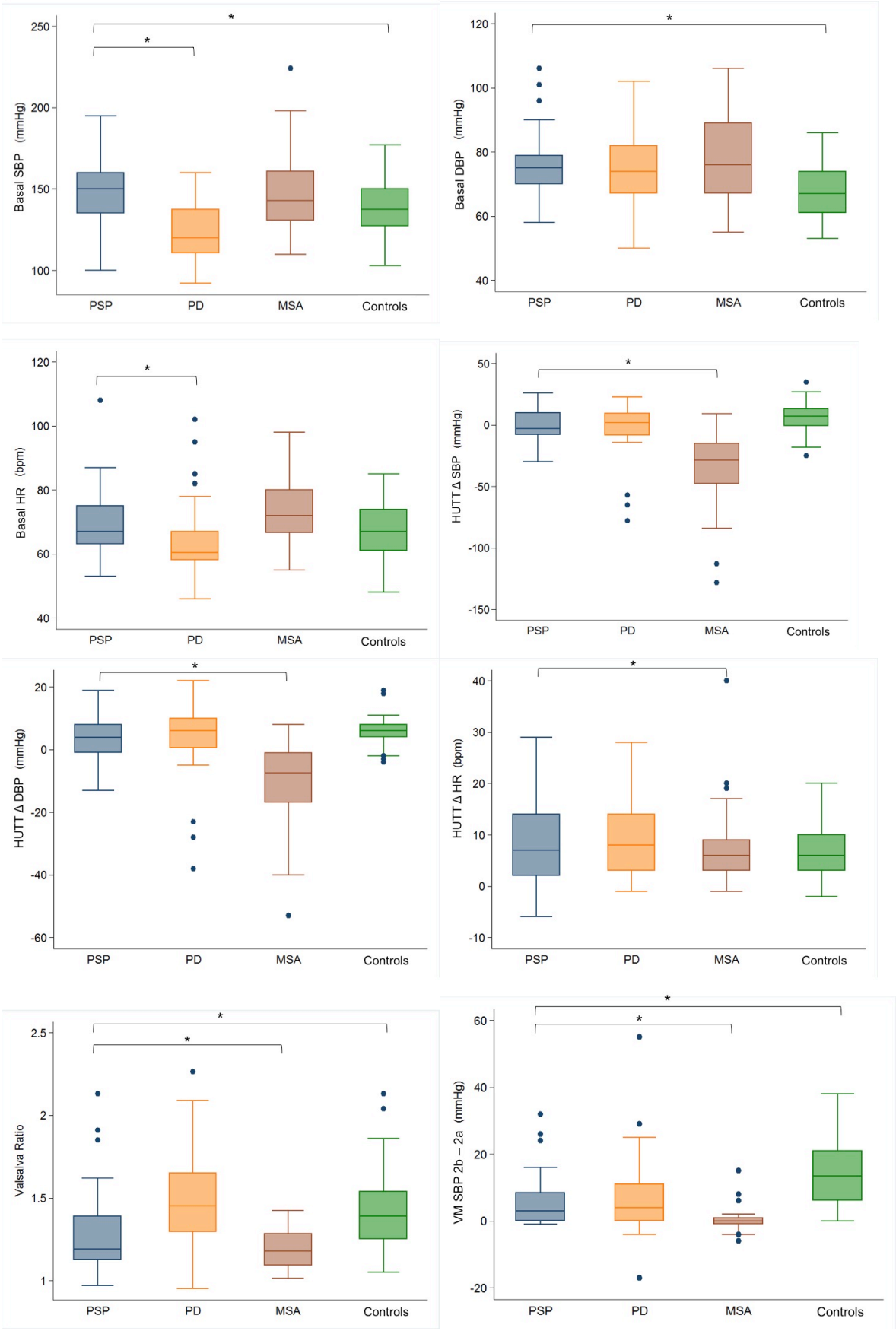
Data are expressed as mean ± standard deviation or number (%) unless otherwise specified.
[a] Data expressed as median (range).
* $p < 0.05$ vs PSP
Legend: PSP = progressive supranuclear palsy; PD = Parkinson's disease; MSA = multiple system atrophy; *N* = number of subjects; BMI = body mass index; PSPRS = PSP rating scale; H&Y = Hoehn and Yahr stage; LEDD = levodopa equivalent daily dose

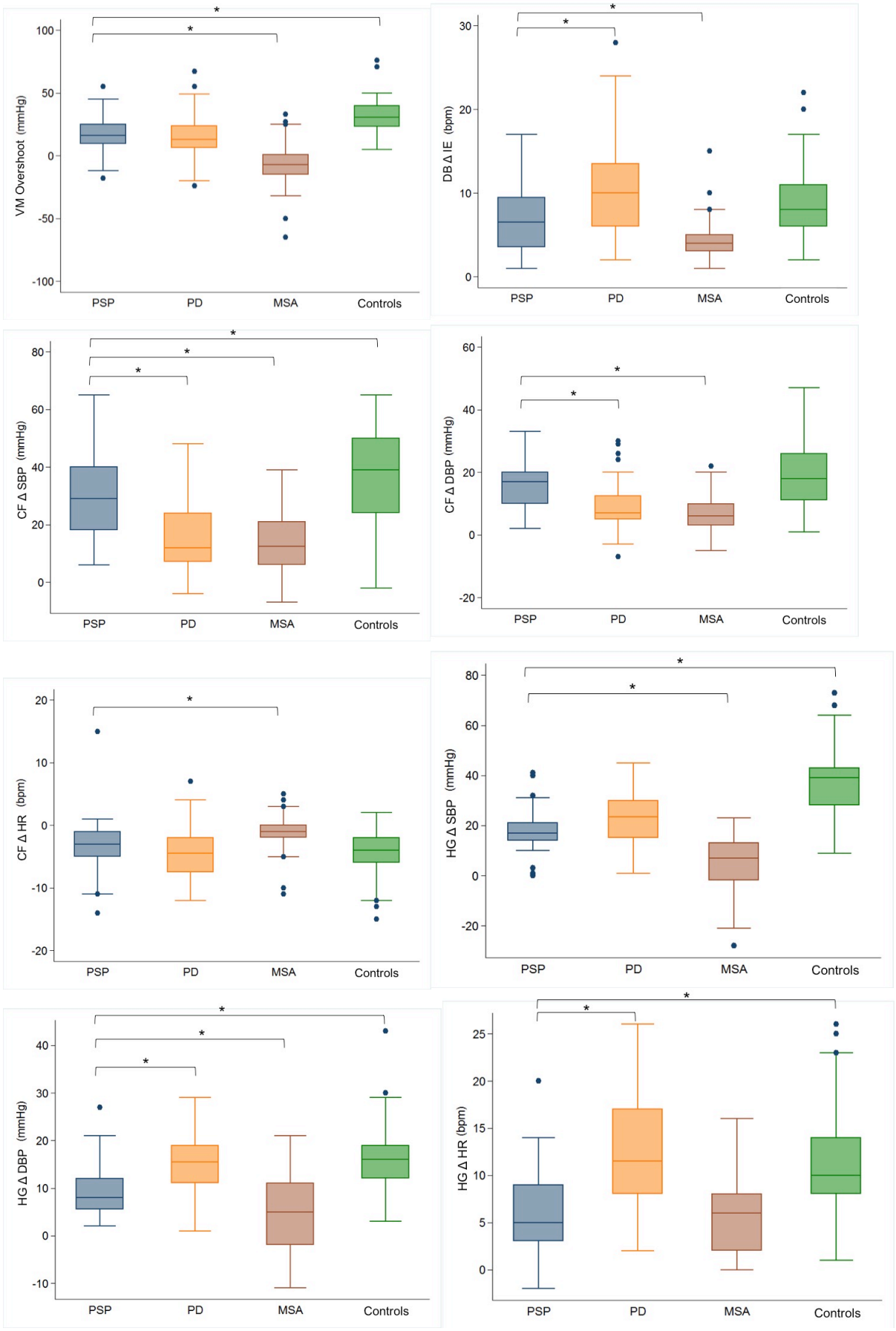
Table 2_Cardiovascular reflex test results

	PSP	PD	MSA	Controls
Basal SBP (mmHg)	149 ± 20	124 ± 18*	149 ± 26	138 ± 18*
Basal DBP (mmHg)	76 ± 10	75 ± 12	78 ± 15	68 ± 8*
Basal HR (bpm)	69 ± 11	64 ± 12*	73 ± 10	66 ± 9
Head-up tilt test	*n = 35*	*n = 40*	*n = 40*	*n = 34*
Δ SBP (mmHg)	-1 ± 15	-3 ± 21	-34 ± 30*	6 ± 12
Δ DBP (mmHg)	3 ± 8	4 ± 12	-11 ± 14*	6 ± 5
Δ HR (bpm)	8 ± 7	9 ± 7	7 ± 7*	7 ± 5
OH (n, %)	5 (14%)	3 (7.5%)	25 (62.5%)	1 (3%)
Neurogenic OH (n, %)	0	3 (7.5%)	24 (60%)	0
Delayed OH (n, %)	0			
Valsalva manoeuvre	*n = 32*	*n = 39*	*n = 37*	*n = 34*
VR	1.30 ± 0.27	1.48 ± 0.29	1.19 ± 0.12*	1.45 ± 0.26*
Δ BP IIb-IIa (mmHg)	7 ± 9	7 ± 12	0 ± 4*	15 ± 12*
Overshoot (mmHg)	17 ± 16	15 ± 20	-8 ± 19*	31 ± 16*
Deep breathing	*n = 32*	*n = 40*	*n = 37*	*n = 34*
Δ IE (bpm)	7 ± 4	11 ± 6*	4 ± 3*	9 ± 5
IE ratio	1.10 ± 0.07	1.17 ± 0.11*	1.06 ± 0.04*	1.14 ± 0.08
Cold face	*n = 35*	*n = 40*	*n = 38*	*n = 33*
Δ SBP (mmHg)	31 ± 16	15 ± 13*	15 ± 11*	37 ± 16*
Δ DBP (mmHg)	16 ± 7	9 ± 8*	8 ± 6*	19 ± 11
Δ HR (bpm)	-3 ± 5	-4 ± 4	-1 ± 3*	-4 ± 4
Handgrip	*n = 32*	*n = 38*	*n = 31*	*n = 33*
Δ SBP (mmHg)	18 ± 10	23 ± 10	4 ± 12*	38 ± 15*
Δ DBP (mmHg)	10 ± 6	15 ± 7*	5 ± 7*	17 ± 8*
Δ HR (bpm)	6 ± 5	12 ± 6*	5 ± 4	12 ± 6*

Data are expressed as mean ± standard deviation unless otherwise specified.
* $p < 0.05$ vs PSP
Legend: PSP = progressive supranuclear palsy; PD = Parkinson's disease; MSA = multiple system atrophy; *n* = number of tests available; SBP = systolic blood pressure; DBP = diastolic blood pressure; HR = heart rate; OH = orthostatic hypotension; VR = Valsalva ratio; Δ = changes compared to basal values.

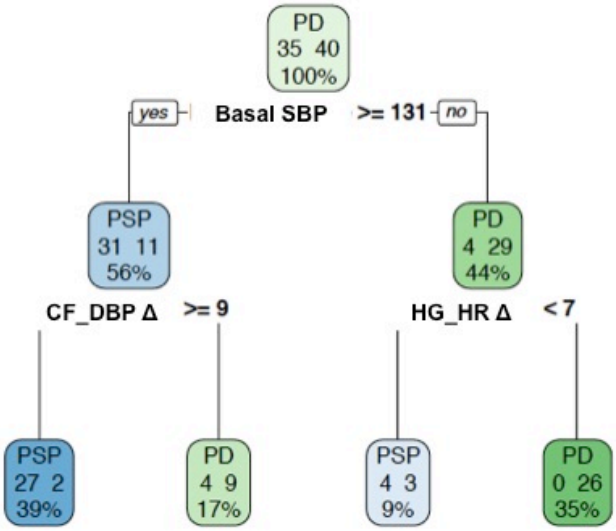
Figure 1_ Cardiovascular reflex test results





Legend: see legend to Table 2.

Figure 2_Results of discriminant analysis



Legend: PD = Parkinson's disease; PSP = progressive supranuclear palsy; SBP = systolic blood pressure; DBP = diastolic blood pressure; HR = heart rate; CF = cold face test; HG = handgrip test; Δ = changes during the test compared to basal values.

DISCUSSION

This study evaluated cardiovascular autonomic function in a cohort of patients with PSP and other neurodegenerative disorders (PD and MSA) as well as sex and age-matched controls.
CRTs were performed according to standardized procedures in a controlled setting to minimize the role of possible confounding factors. Importantly, BP changes during the tests could be estimated precisely thanks to the continuous beat-to-beat monitoring of BP, which allowed the correct classification of OH as neurogenic or non-neurogenic based on BP changes occurring during the Valsalva manoeuvre that was performed in sequence after the HUTT.

In this study, 5 out of 35 PSP patients (14%) presented OH, however none of them had neurogenic OH (that is, OH was associated with a normal VM in all patients). On the contrary, OH was neurogenic in all PD and MSA patients. This finding is of relevance for several reasons: firstly, from a pathophysiological point of view, it highlights the importance of a thorough evaluation of cardiovascular autonomic function by means of CRTs in a parkinsonian patient with OH. The correct classification of OH as neurogenic or non-neurogenic provides additional information that may help in the diagnostic process of such patients. Secondly, considering that treatment significantly varies between these two conditions, it is important to tailor therapy.
This study is in line with the only former work that evaluated the presence of NOH in PSP (finding none)(Van Gerpen *et al.*, 2019). Regarding overall OH prevalence, the 14% observed in this study is similar to the majority of previous studies (prevalence 8-16%)(Schmidt *et al.*, 2008; Schmidt, Berg, *et al.*, 2009; Schmidt, Herting, *et al.*, 2009; Reimann *et al.*, 2010; Oliveira *et al.*, 2019). It is also not in contrast with those that did not find OH in PSP(Kimber *et al.*, 2000; Kikkawa *et al.*, 2003); indeed, secondary OH resolves if causal agent is removed, and it is likely that the strict patient selection and test standardization applied in these studies lead to such results. The higher OH frequency reported in few studies is possibly related to methodological factors, particularly the possible confounding role of medications (not specified whether they were discontinued)(Wenning *et al.*, 1999; Bae *et al.*, 2009).

On the whole, cardiovascular responses to the stimuli administered during CRTs were preserved in PSP. However, the comparison with CRTs results from sex and age-matched controls highlighted significant differences in the BP basal values (higher in PSP) and responses to the VM, handgrip test and SBP response to cold face test, which were all slightly blunted in PSP. These data may indicate an impairment of cardiovascular sympathetic function. However the lack of significant differences in BP drop on HUTT, together with the absence of neurogenic OH in this group as mentioned earlier, suggest that such sympathetic impairment in PSP is mild and not clinically significant.

Regarding cardiovagal function, only the Valsalva ratio resulted significantly different compared to controls, whereas other indexes of heart rate variability (heart rate response to HUTT, deep breathing and cold face test) were normal. It should be noticed that the mean value for the Valsalva ratio in PSP was 1.30, which is still considered within normal limits for this age range. Therefore, the results of this study support an overall preserved cardiovagal function in PSP.

In this study, disease duration and severity (expressed as H&Y stage) failed to significantly influence CRTs results. The few statistically significant values were not consistent across all tests and therefore should not be considered clinically relevant. However, to better estimate the effect of disease duration and disease severity a prospective study with multiple observations of the same patient should be performed. In other words, the design of this study might not be suitable to evaluate this aspect of cardiovascular autonomic function in PSP.

Previous studies with autonomic testing described sympathetic impairment in PSP(Sandroni *et al.*, 1991; van Dijk *et al.*, 1991; Gutrecht, 1992; Friedrich *et al.*, 2008, 2010; Schmidt *et al.*, 2008; Schmidt, Berg, *et al.*, 2009; Schmidt, Herting, *et al.*, 2009; Reimann *et al.*, 2010; Nojszewska *et al.*, 2019), however they showed also significant differences in parasympathetic parameters, in contrast to this study. Both parasympathetic and sympathetic responses were intact in several other works(Brefel-Courbon *et al.*, 2000; Kimber *et al.*, 2000; Holmberg *et al.*, 2001; Deguchi *et al.*, 2002; Kikkawa *et al.*, 2003). Factors related to normative values or controls employed in each study may concur to provide these different results. Indeed, no severe autonomic failure was observed in any study.

A certain degree of autonomic impairment is consistent with neuropathological findings in areas of the central autonomic network. One neuropathological study on 17 PSP demonstrated consistent abnormalities in several autonomic nuclei located in the brainstem, namely the medial and parabrachial nuclei, the gigantocellular reticular nucleus, the raphes magnus and raphes obscurus nuclei and intermediate reticular zone, all of which play a role in the regulation of cardiovascular function and/or micturition. Such abnormalities were observed even in cases with short disease duration, however the correlation with autonomic symptoms in life in these patients is unknown(Rüb *et al.*, 2002). The intermediolateral column in the spinal cord was found to be spared(Iwasaki *et al.*, 2007) or moderately affected (39% nerve cell loss) by the neurodegenerative process(Vitaliani *et al.*, 2002), yet none of the patients in the latter study presented signs of autonomic failure in life. Significant pathology was not found in spinal ganglia but sympathetic ganglia were not specifically assessed(Wakabayashi *et al.*, 2010). Therefore, it is possible that the autonomic findings observed in this study are secondary to a supraspinal involvement of the autonomic nervous system by tau pathology. Further studies are needed to better elucidate pathophysiological mechanisms behind autonomic dysfunction in PSP.

The comparison between PSP and other neurodegenerative parkinsonian syndromes is of interest as differential diagnosis may sometimes be difficult. Characteristics of non-motor features including cardiovascular autonomic function may indeed help in the diagnostic process in certain settings(Baschieri *et al.*, 2015).

PSP and PD showed similar responses to HUTT and VM, suggesting that baroreflex function as explored by these tests is comparable between such patients. The lower heart rate variability observed in PSP at deep breathing is not confirmed at other tests (VM and cold face test). It is likely that the reduced heart rate variability at deep breathing reflects a difficulty of PSP patients in performing controlled voluntary breathing cycles compared to PD, rather than cardiovagal impairment. As a matter of fact, in the study by De Bruin and colleagues, PSP patients had difficulty performing spirometry while nocturnal oxygen saturation was normal(De Bruin *et al.*, 1996). The Authors concluded that PSP might have a supranuclear impairment of respiratory control (i.e. voluntary respiratory acts), whilst maintaining automatic control of breathing. Therefore, in this study, sympathetic and parasympathetic functions seem overall comparable between PSP and PD. As mentioned in the introduction, previous studies on this topic were inconclusive. Indexes of sympathetic function were either equal(Sandroni *et al.*, 1991; Deguchi *et al.*, 2002; Kikkawa *et al.*, 2003; Bae *et al.*, 2009; Schmidt, Herting, *et al.*, 2009), more(van Dijk *et al.*, 1991; Friedrich *et al.*, 2008) or less compromised(Holmberg *et al.*, 2001; Schmidt *et al.*, 2008; Reimann *et al.*, 2010) in PSP compared to PD. Likewise, parasympathetic function in PSP was more impaired (Sandroni *et al.*, 1991; van Dijk *et al.*, 1991) or comparable(Holmberg *et al.*, 2001; Deguchi *et al.*, 2002; Kikkawa *et al.*, 2003; Schmidt *et al.*, 2008; Schmidt, Herting, *et al.*, 2009; Reimann *et al.*, 2010) to PD. However the majority of studies confirmed our results, and it is likely that those that found a difference were observing the confounding effect of some other clinical variable such as disease duration and disease severity for example.

The remaining tests provided somewhat conflicting results. The lower increase in BP and HR at handgrip test in PSP compared to PD, possibly suggesting an impairment of peripheral sympathetic efferent fibres, is not consistent with the higher increase (in PSP compared to PD) of the same parameters observed at the cold face test. It should be noted that the cold face and handgrip tests, while eventually culminating both in the activation of peripheral efferent sympathetic fibres, are triggered by the integration of stimuli from chemoreceptors, mechanoceptors, nociceptors and central commands, therefore several factors may impact the final responses(Freeman, 2006; Khurana, 2007). Additionally, there is a considerable degree of variability between subjects for these tests, particularly for the handgrip test where it could be difficult to standardize the muscular effort in patients with motor and cognitive impairments(Freeman, 2006).

Based on the CRTs results of this study, there doesn't seem to be a single test or particular pattern of dysfunction that allow to distinguish PSP from PD. The discriminant analysis was performed with the aim of finding whether a combination of results obtained from CRTs may help in identifying the correct diagnosis (PSP versus PD). The parameters that mostly differentiated these two groups were SBP at supine rest, DBP

response to cold face test and heart rate response to handgrip test. These findings suggest that PSP and PD may actually present a different involvement of autonomic structures outside the baroreflex arch. Further studies are needed to better elucidate this interesting topic.

MSA patients showed significantly more compromised cardiovascular responses to all CRTs compared to PSP. This is expected and in line with the severe autonomic failure that characterizes this neurodegenerative condition. Consequently, severe cardiovascular autonomic failure (usually with NOH) points towards MSA diagnosis and may be support in the differential diagnosis between these two conditions. Previous studies confirmed these findings(Brefel-Courbon *et al.*, 2000; Kimber *et al.*, 2000; Holmberg *et al.*, 2001).

The main limitations of this study is the lack of neuropathological confirmation, however only patients that were followed up prospectively or had complete medical records available that did not show any red flag for other diagnosis were included. Another limitation was the low number of patients, which was nevertheless higher than the majority of the studies previously reported.

In conclusion, PSP presented mild cardiovascular adrenergic impairment not clinically significant and preserved cardiovagal function. A minority of PSP patients had non-neurogenic OH. PSP showed similar cardiovascular responses to PD in the tests that explored the baroreflex arch whereas some differences could be found at cold face and handgrip tests. All cardiovascular responses were significantly more compromised in MSA than PSP.

Section III

CARDIOVASCULAR AUTONOMIC FUNCTION IN PROGRESSIVE SUPRANUCLEAR PALSY

Circadian rhythm of blood pressure and heart rate

BACKGROUND

Twenty-four hour blood pressure (BP) monitoring in healthy subjects demonstrates that mean BP values at night-time fall by 10-20% compared to mean daytime values (dipping pattern). The same happens for heart rate (HR).

A loss of the expected nocturnal BP fall (either a reduced decrease or a non-dipping or a reverse-dipping pattern) may be found in certain pathological states such as essential hypertension, which is the most common cause. A loss of the dipping pattern may also be observed in patients with neurogenic orthostatic hypotension (OH) and supine hypertension(Fanciulli *et al.*, 2018).

So far, only one group of researchers explored the 24-hour BP profile in progressive supranuclear palsy (PSP). The Authors examined the 24-hour BP profile by means of ambulatory monitoring and found night-time hypertension in 36%, a reduction of the expected BP night fall in 40% and a reverse dipping pattern in 8% of PSP patients(Schmidt, Berg, *et al.*, 2009; Reimann *et al.*, 2010). Statistical analysis showed a significant correlation between increased BP night-time values and presence of OH at head-up tilt test (HUTT) (74% of patients with a paradox nocturnal blood pressure increase also presented OH at HUTT, whereas only 11% of patients without OH had a reversed nocturnal BP profile)(Schmidt, Berg, *et al.*, 2009). Nonetheless, it was not specified whether OH, and as a consequence supine hypertension, had a neurogenic origin. In other words, it is not possible to rule out other causes for these findings (i.e. essential hypertension and OH secondary to medications) even though patients treated with anti-hypertensive medications were excluded from the study which makes this hypothesis unlikely.

The aim of this study was to evaluate the circadian rhythm of BP and heart rate (HR) in a cohort of PSP patients. In addition, we aimed to assess the interaction between the sleep-wake cycle and cardiovascular parameters in patients with PSP by calculating mean BP and HR values in each sleep stage and wake (state-dependent modulation).

MATERIALS AND METHODS

Participants

Fifteen patients with PSP diagnosed according to current consensus(Höglinger *et al.*, 2017) criteria were recruited (13 prospectively and 2 retrospectively). One male patient recruited prospectively removed most part of the recording set during the first night of recording and did not complete the study protocol and therefore was excluded. The patients did not have other severe medical conditions that could have affected study results. Anti-hypertensive medications were discontinued from the night before when considered safe to do so. All patients gave their written informed consent to participate in the study.

Study protocol

BP) and HR were monitored continuously as part of the study protocol for sleep/wake cycle and circadian rhythm of body core temperature described in the previous sections.

Systolic and diastolic BP (SBP, DBP) and HR were monitored beat-to-beat with Portapres portable recorder (Portapres® Model-2, Finapres Medical Systems, Amsterdam, The Netherlands) during the first 24 hours. Over the subsequent 24 hours BP and HR were recorded with a standard upper arm cuff every 20 minutes during daytime and 30 minutes during night-time (OnTrak 90227 Ambulatory Blood Pressure Spacelabs Healthcare Ltd). During the study, subjects were allowed to sleep ad libitum, living in a temperature (24 ± 1 °C) and humidity (40-50%) controlled room, lying in bed except when eating, in a light-dark schedule (dark period: 23:00 - 7:00). The subjects were placed on a 1.800 kcal/day diet, divided into three meals (8:00, 12:00, 18:00) and three snacks (10:00, 16:00, 23:00). From midnight preceding the monitoring, subjects were instructed to avoid alcohol and caffeinated beverages and to abstain from smoking.

Cardiovascular parameters

Day-night pattern of SBP, DBP, HR

We calculated SBP, DBP and HR daytime (7:00 – 23:00) and night-time (23:00 – 7:00) mean values, and nocturnal decline of these parameters determined by the difference between night-time and daytime values (ΔSBP, ΔDBP, ΔHR).

24-hr circadian rhythm of SBP, DBP, HR

For the data registered with Portapres portable recorder, we were able to analyse rhythmicity by evaluating the time series for SBP, DBP and HR according to the single cosinor method, using a computerized procedure (Mojón *et al.*, 1992). In brief, this procedure elaborates the raw curves obtained across the 24-hour period using a specific mathematical curve-fitting method (cosine curve). The procedure then determines whether or not there is a rhythm with a 24-hour period and evaluates the following parameters within their 95% confidence limits: (1) mesor (Midline Estimating Statistic of Rhythm), that is the mean value of the cosine function; (2) amplitude, defined as the difference between the maximum value measured at the acrophase and the mesor of the cosine curve used to approximate the rhythm; and (3) acrophase, defined as the interval between midnight hour (reference time) and time of highest value of the cosine function. For each patient we analysed the 24-h rhythmicity of a single day, starting at 11:00.

State-dependent analysis of SBP, DBP, HR

For the data registered with Portapres portable recorder, we determined the mean value of SBP, DBP and HR in wake and each sleep stage (NREM stage 1, 2, 3 and REM sleep) over the 24-hr period.

Statistical analysis

Descriptive analysis was performed with SPSS Statistics (version 25).

RESULTS

Demographics and clinical characteristic of the PSP patients are reported in **Table 1**. Fourteen patients were studied, 6 males and 8 females with mean age 74.8 ± 3.9 years. The majority of patients had a diagnosis of hypertension and were being treated with anti-hypertensive medications. These medications were discontinued prior to recording in 5 patients with constant monitoring of the values to exclude a rebound effect. A summary of mean BP and HR values during day- and night-time are reported in **Table 2**. The mean daytime BP met the criteria for hypertension in only 2 patients, and was only systolic in both cases. A dipping BP pattern was observed only in 2 patients, whereas 7 patients had a reduced dipping or non-dipping BP pattern. The remaining 5 patients had a reverse dipping BP pattern.

Continuous beat-to-beat BP monitoring was available for 5 patients only. The rest of the patients were not able to perform this recording due to technical difficulties. Data from the circadian rhythm analysis are reported in **Table 3**. A rhythm was detected for all patients for both BP and HR. For BP, mesor was increased in 3 patients, amplitude was similarly reduced in 3 patients and acrophase was postponed in 1 patient and advanced in 3 (compared to expected values). Regarding HR, mesor was normal for all patients, amplitude was reduced in 2 patients and acrophase was postponed in 2 patients. The alterations in HR were independent from those in BP. Results from the state-dependent analysis are also reported in **Table 3**. State-dependent values were available for 4 patients only and of these, only 2 reached REM stage during the night (this was the first night of the 48-h recording of the study protocol). SBP did not fall as it would have been expected in NREM stage 1 and 2 but decreased in NREM stage 3 and REM. Instead, DBP showed a preserved decrementing trend through sleep stages as well as HR.

Table 1_Demographics and clinical characteristics of PSP patients

	PSP
N	14
Age (years)	74.8 ± 3.9
Males (n, %)	6 (43%)
BMI (Kg/m^2)	28.0 ± 3.6
Disease duration (years)	5.8 ± 2.6
PSPRS	51.3 ± 10.7
Anti-hypertensive medication (n, %)	11 (78%)
Withdrawn for study	*5 /11*

Legend: PSP = progressive supranuclear palsy; *N* = number of patients; BMI = body mass index; PSPRS = PSP rating scale.

Table 2_Day- and night-time profile of blood pressure and heart rate in PSP (standard arm-cuff)

	PSP *(n = 14)*
SBP day (mmHg)	126.5 ± 14.6
SBP night (mmHg)	123.2 ± 14.8
SBP Δ (mmHg)	-3.3 ± 10.1
DBP day (mmHg)	73.1 ± 6.8
DBP night (mmHg)	70.9 ± 6.9
DBP Δ (mmHg)	-2.3 ± 5.4
HR day (bpm)	74.9 ± 5.9
HR night (bpm)	69.4 ± 7.0
HR Δ (bpm)	-5.4 ± 4.7
BP pattern at night-time	
Dipping (n, %)	2 (14%)
Reduced/non-dipping (n, %)	7 (50%)
Reverse dipping (n, %)	5 (36%)

Legend: PSP = progressive supranuclear palsy; SBP = systolic blood pressure; DBP = diastolic blood pressure; HR = heart rate; bpm = beats per minute; Δ = difference (night – day).

Table 3_Circadian rhythm and state-dependent modulation of BP and HR in PSP (beat-to-beat monitoring)

	PSP *(n = 5)*
SBP	
mesor (mmHg)	161.2 ± 28.1
amplitude (mmHg)	9.8 ± 4.6
acrophase (°)	123 ± 58
DBP	
mesor (mmHg)	80.4 ± 10.1
amplitude (mmHg)	4.0 ± 2.8
acrophase (°)	136 ± 74
HR	
mesor (bpm)	73.2 ± 4.4
amplitude (bpm)	5.6 ± 3.0
acrophase (°)	288 ± 43
SBP	
W (mmHg)	153.8 ± 20.5
N1 (mmHg)	154.0 ± 30.2
N2 (mmHg)	155.1 ± 29.1
N3 (mmHg)	138.1 ± 22.0
R (mmHg)	141.1 ± 25.1
DBP	
W (mmHg)	78.3 ± 5.4
N1 (mmHg)	74.5 ± 9.0
N2 (mmHg)	74.4 ± 10.1
N3 (mmHg)	67.6 ± 7.0
R (mmHg)	71.6 ± 10.9
HR	
W (bpm)	75.6 ± 6.5
N1 (bpm)	62.7 ± 0.8
N2 (bpm)	61.7 ± 1.6
N3 (bpm)	60.5 ± 0.9
R (bpm)	61.9 ± 0.8

Legend: PSP = progressive supranuclear palsy; SBP = systolic blood pressure; DBP = diastolic blood pressure; HR = heart rate; bpm = beats per minute; W = wake; N1 = NREM sleep stage 1; N2 = NREM sleep stage 2; N3 = NREM sleep stage 3; R = REM sleep.

DISCUSSION

This is the first study to assess the 24-h BP and HR profile in PSP under controlled environmental conditions. The majority of the patients included in this study had a diagnosis of essential hypertension and received treatment for it. We were able to discontinue such medications in half of the patients so the majority of the patients studied were not under the direct influence of such medications although it cannot be excluded that the effect of chronic treatment may have played a role. Only 2 patients presented the expected dipping BP pattern, whereas the rest had either a reduced/non-dipping pattern or a reverse dipping pattern. Therefore the 24-h BP profile in PSP was abnormal in 86% of the patients for the absence of the expected BP fall.

This finding may be due to essential hypertension and has been related to increased cardiovascular risk and mortality both in hypertensive and normotensive subjects(Grimaldi *et al.*, 2013). The clinical relevance of this finding resides in the potential therapeutic implication as restoring the normal nocturnal BP decline through medication ingested at the appropriate time may be indicated in such patients. PSP had been previously associated with essential hypertension particularly in the pre-symptomatic phase. One retrospective study found that a history of hypertension on medical records or reported by patients was present in 80% of the PSP patients, a number significantly higher than what encountered in other parkinsonian disorders(Ghika and Bogousslavsky, 1997). Nevertheless, subsequent works including one with pathological confirmation failed to confirm such results, finding that the prevalence of hypertension in PSP was similar to the general population(Fabbrini *et al.*, 1998; Colosimo *et al.*, 2003). However, Sibon et al pointed out the possibility that cases with "mixed" vascular and neurodegenerative pathologies might be more frequent than expected and might have been missed by studies focusing on "pure" neurodegenerative PSP(Sibon *et al.*, 2004). In our cohort, a previous diagnosis of essential hypertension was very frequent (78%).

Compared to the PSP cohort previously studied with 24-h ambulatory BP monitoring(Schmidt, Berg, *et al.*, 2009; Reimann *et al.*, 2010), we found a higher rate of reduced and reverse dipping pattern. Similarly in contrast, no patient had nocturnal hypertension. In these previous studies, patients treated with anti-hypertensive medications were excluded and this may represent a reason for the discrepancy. It also suggests that the altered 24-h BP pattern observed in PSP may not be only attributable to essential hypertension.

Schmidt and colleagues suggested that these findings may represent a marker of cardiovascular dysautonomia. Indeed, impairment of cardiovascular autonomic function may manifest with supine and nocturnal hypertension in patients with neurogenic OH. However, all the patients in our study performed cardiovascular reflex tests just before starting the monitoring and only 1 presented OH which was however associated with a normal Valsalva manoeuvre, suggesting a non-neurogenic (i.e. secondary) origin for OH. Therefore, ascribing these findings only to cardiovascular autonomic failure may not be appropriate.

New insights may come from the concomitant study of sleep and BP monitoring throughout the 24 hours. As shown in the previous section, PSP patients presented a profound sleep loss during the night-time, with frequent and sometimes protracted awakenings. It is possible that such sleep fragmentation and deprivation may prevent the expected fall and induce the reduced or reverse dipping BP pattern observed. Another factor that could contribute to an abnormal nocturnal BP profile is obstructive sleep apnea (OSA). OSA, as previously introduced in Section I, is characterized by repetitive episodes of complete (apnoea) or partial (hypopnea) upper airway obstruction during sleep, usually associated with snoring. Each episode leads to oxygen desaturation that usually terminate with a brief arousal from sleep, causing sleep fragmentation(Sands *et al.*, 2018). OSA leads to sympathetic overactivity as both an acute effect of the apnoea and a chronic effect resulting from remodelling of the autonomic nervous system balance(Calandra-Buonaura *et al.*, 2016). Indeed, our patients with a dipping BP pattern did not have OSA, while 6 out of 12 with a reduced/non-dipping/reverse dipping pattern had OSA (this data was not available for further 3 of these 12 patients, while the remaining 3 subjects did not have OSA). Therefore, it is likely that this breathing disorder contributed to the abnormal BP profile in our PSP patients.
Restless legs syndrome (RLS) and periodic limb movements of sleep (PLMS), similarly discussed in Section I, have also been associated with hypertension(Chiaro and Manconi, 2019), possibly as a consequence of an impaired autonomic control of the cardiovascular system towards a sympathetic predominance(Calandra-Buonaura *et al.*, 2016). PLMS occur with transitory changes in HR, BP an EEG that, recurring repetitively overnight may eventually lead to persistent increase in sympathetic tone. Alternatively, cardiovascular dysautonomia may be a consequence of sleep alterations induced by RLS, namely insomnia and short sleep duration. Indeed the lack of profound daytime sleepiness which would have been expected given the sleep loss is a supportive clinical feature of RLS(Allen *et al.*, 2014), suggesting that RLS may represent an hyperarousal condition. Our PSP patients with dipping BP profile did not have PLMS however they reported RLS. Among those with a pathological BP profile, 4 out of 12 had RLS and 8 out of 12 had PLMS (index > 10). Therefore, in our PSP patients, PLMS may represent a contributing factor to BP alterations whereas the role of RLS without PLMS is less defined.
State-dependent analysis showed a partial loss of the state-dependent modulation for SBP while the modulation seemed preserved for other parameters (DBP and HR). To better clarify the meaning of these results, a larger sample that includes patients with and without a diagnosis of essential hypertension and matched controls is warranted.

Limitations of this study are the small number of patients, in particular those that were able to perform the continuous beat-to-beat BP monitoring and the lack of neuropathological confirmation. As discussed, the majority of patients were being treated for hypertension, however we were able to discontinue drugs for the monitoring in most cases. We did not have matched controls to compare our results.

CONCLUSIONS

CONCLUSIONS

The aims of this study were to describe and evaluate the association between sleep, the circadian system and autonomic function in a cohort of patients with progressive supranuclear palsy (PSP).

The main findings of the current study are:

- PSP patients complain of poor sleep very often, mainly related to inability to fall asleep or maintain sleep while excessive daytime sleepiness seems quite uncommon. Main polysomnographic findings of PSP patients are a reduced total sleep time, increased wake after sleep onset, frequent awakenings, increased light sleep (stage NREM 1) and reduced REM sleep duration. During daytime patients had very short naps with a median duration of less than 1 hour. When considering the total amount of sleep/24 h (summing the dark + light periods), median total sleep time was still lower than the recommended amount of sleep for the elderly. Therefore, even if PSP had sleep loss during the night, they did not sleep during the daytime as a compensating mechanism, yielding to a condition of profound sleep deprivation across the 24 hours.
- REM sleep behaviour disorder (RBD) was found in 15% and REM sleep without atonia in 77% (median 16% of total REM). RBD should not be considered an exclusion criterion for PSP diagnosis although it is manifested in a minority of patients.
- Approximately half of the patients had restless legs syndrome and more than half had severe periodic limb movements in sleep which however did not seem to significantly affect sleep structure.
- PSP presented the physiological night-time decrease of body core temperature (BcT). Comparing PSP results to those of the control group, PSP had significantly higher mean day and night values, but similar BcT Δ. Therefore the expected variability of BcT (night-time BcT fall) was present in PSP as it is in normal subjects. Regarding the circadian rhythm study, all PSP patients presented BcT rhythmicity. Compared to controls, PSP had similar acrophase and amplitude, whereas mesor was significantly higher. These data seem to suggest that PSP have preserved circadian rhythmicity (expressed by the acrophase) but may have an impairment of the homeostatic control (i.e. increased mesor) of thermoregulation.
- PSP maintained the physiological state-dependent modulation of BcT between wake, NREM and REM sleep however presented higher mean values in each state. This may reflect an impairment of sympathetic function, particularly an inability to reduce sympathetic function during sleep or it may be a consequence of the altered sleep structure (sleep deprivation and higher representation of light sleep) demonstrated by the PSP.
- PSP presented mild cardiovascular adrenergic impairment not clinically significant and preserved cardiovagal function. A minority of PSP patients had non-neurogenic orthostatic hypotension. PSP showed similar cardiovascular responses to Parkinson's disease in the tests that explored the baroreflex arch whereas some differences could be found at cold face and handgrip tests. All cardiovascular responses were significantly more compromised in multiple system atrophy than PSP.

- The majority of our PSP patients had a diagnosis of essential hypertension. Only 2 patients presented the expected dipping BP pattern, whereas the rest had either a reduced/non-dipping pattern or a reverse dipping pattern. This finding may be related to hypertension but also to disturbed sleep and possible concomitant effect of sleep disorders like obstructive sleep apnoea and periodic limb movement of sleep.
- State-dependent analysis showed a partial loss of the state-dependent modulation for systolic blood pressure while the modulation seemed preserved for other parameters (diastolic blood pressure and heart rate).

In conclusion, this study showed that PSP presented abnormalities of sleep, circadian rhythms and cardiovascular autonomic function that are likely to be closely linked one to another. Further studies should be designed to investigate the potential effect of interventions (treating periodic limb movements of sleep and other sleep disorders, hypertension, etc…) on the association between these features. A better understanding of these topics are needed to improve clinical practice not only in the diagnostic process but also to personalize treatment for each patient and improve quality of life.

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